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DEVELOPMENT OF AXILLARY BUDS ON FRUITING BRANCHES OF PIMA AND UPLAND COTTON [1]

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INTRODUCTION

The cotton plant produces two distinct types of branches, which occupy different positions and have different structures and functions. These are the upright "limbs" or vegetative branches, which do not produce flowers or bolls, and the fruiting branches, which assume horizontal positions and on which are developed the floral buds. Cook [2] has shown that in addition to the bud that serves to continue the growth of the shoot, each node of the main stalk produces two other buds, one in the axil of the leaf and another to the right or left of the axil, called an extra-axillary bud. The axillary buds usually remain dormant, but may be developed into vegetative branches when conditions for luxuriant growth are favorable. The fruiting branches arise from the extra-axillary buds on the main stalk and on vegetative branches.

The fruiting branches also produce two buds at each node, corresponding to the two types on the main stem. These are a flower bud, which appears between the bases of the stipules, and an axillary bud, which is formed in the axil of the leaf. It has been observed that the axillary buds on the fruiting branches of Egyptian cotton differ somewhat in morphology and behavior from those on the upland type. These distinctions and their possible relationship to cultural problems and studies in bud shedding are presented in this paper.

COMPARISON OF AXILLARY BUDS ON EGYPTIAN AND UPLAND COTTONS

Under ordinary conditions of growth a greater part of the axillary buds on the fruiting branches of upland cotton remain dormant, but in cases of exceptional luxuriance, or when the terminal growth of the branches or main stem is injured, a considerable number of them may undergo further development and produce short vegetative branches. (Fig. 1.) [3]

In Egyptian cotton the axillary buds on the fruiting branches seldom if ever remain dormant for any great length of time. They emerge from their positions in the axils of the leaves as minute triangular buds or "squares," about two or three days after the extra-axillary bud on the same node, and at first in all respects appear

[1] Received for publication July 17, 1930; issued November, 1930.

[2] COOK, O. F. MORPHOLOGY OF COTTON BRANCHES. U. S. Dept. Agr., Bur. Plant Indus. Circ. 109: 11-16, illus. 1913.

[3] The photographs illustrating this paper were made by H. F. Loomis.

like normal fruit buds. They are unlike the axillary buds of upland cotton in that they are usually unattended by a leaflet with stipules. (Fig. 2.) Under ordinary conditions their growth continues for only a few days, and then they drop off or begin to shrivel and finally dry up into minute dark-colored bodies, many of which remain attached to the fruiting branches.

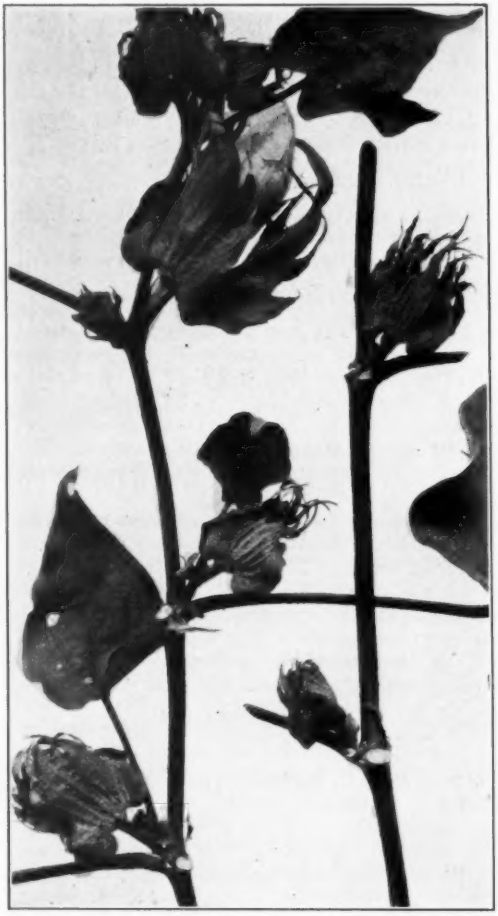

FIGURE 1.—A fruiting branch (in two sections) of Acala (upland) cotton, showing development of axillary buds at every node. This development occurred late in the season, long after the shedding of extra-axillary bolls on the first four nodes, whose positions are shown by the scars. At node 5 the largest bud shown is the extra-axillary, and the small bud to the left is the axillary. At node 6 only the extra-axillary bud is visible

If the plants are especially productive or if the terminal growth of branch or main stem is aborted or injured, a number of the axillary buds may continue development. When this occurs there usually is produced a single boll on a long stem (fig. 3), but occasionally two or more bolls may develop. Some of the boll stems have the appearance of simple pedicels, but many of them show some type of joint and occasionally small bractlike leaves or stipules, which indicate that the "pedicels" comprise shortened branches. Cook[4] points out that in reality three independent elements are represented in such stems — an axillary branch, a fertile branch from the axillary, and the pedicel of the boll, all fused into a simple stem. This type of fusion is carried much farther in Egyptian cotton than in upland cotton. In upland cotton the boll stems invariably have a well-defined joint to indicate that a branch is involved. (Fig. 4.)

[4] COOK, O. F. DIMORPHIC BRANCHES IN TROPICAL CROP PLANTS: COTTON, COFFEE, CACAO, THE CENTRAL AMERICAN RUBBER TREE, AND THE BANANA. U. S. Dept. Agr., Bur. Plant Indus. Bul. 198, 64 pp., illus. 1911.

FACTORS THAT AFFECT DEVELOPMENT OF AXILLARY BUDS

The difference in the behavior of the axillary buds in the two types of cotton is of importance in relation to some of the production problems that are peculiar to certain of the irrigated valleys of the Southwest. In these regions the growing seasons are long, but the cotton plants are frequently forced to undergo periods of severe

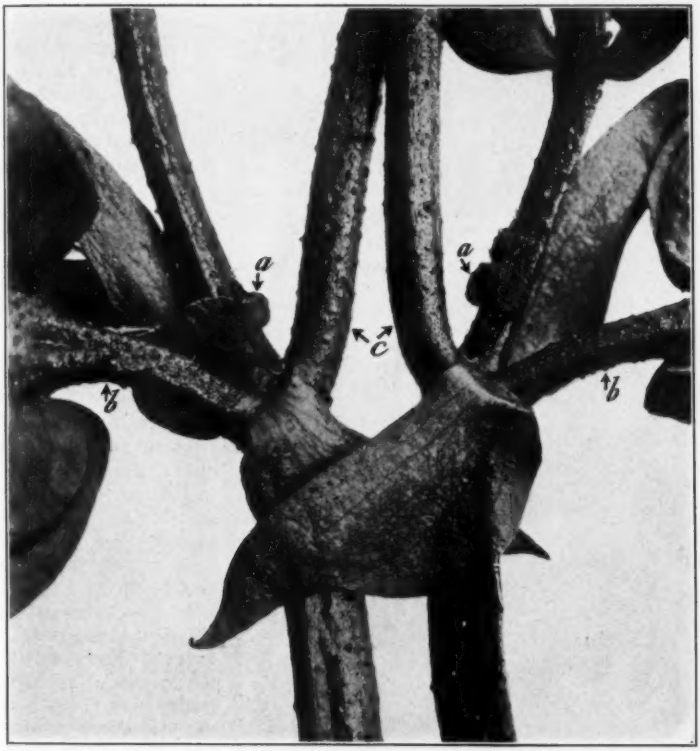

FIGURE 2.—Relative positions of extra-axillary and axillary fruit buds at each node on the fruiting branches of Pima Egyptian cotton plants. The small axillary buds or squares can be seen, at the base of the leaf petioles, as they appear when three or four days old, and are about ready to drop off or shrivel up. It will be noted that these buds usually do not develop the subtending leaf and stipules which characterize this type of bud in upland cotton: *a*, Axillary bud; *b*, pedicel of extra-axillary bud; *c*, petiole of leaf

stress, resulting from improper water relations when high temperatures prevail. Since the axillary buds on the fruiting branches of Egyptian cotton do not remain dormant and ordinarily survive for only a few days, it is apparent that any stimulus that would influence the retention and further development of these buds must be exerted before or during the few days when they are in full vigor of growth.

With upland cotton, on the other hand, the establishment of the proper conditions can apparently stimulate the growth of the dormant axillary buds at any time during the growing season, especially if the plants have previously thrown off a large part of their extra-axillary

fruit. In some of the hot interior valleys of the irrigated region the upland varieties are prone to undergo excessive shedding of buds and young bolls during periods of high temperature, and in some seasons many of the plants may shed all of the bolls and buds that are in the early stages of development during such periods. It has been observed that after climatic conditions have moderated near the close of an especially hot and unfavorable summer many of the upland plants that have undergone excessive shedding may begin to develop at almost every node, these short axillary branches bearing one or more floral buds. (Fig. 5.) Some even emerge from the first nodes near the main axis, where in rare cases a mature boll may be found on the same node.

FIGURE 3.—Axillary and extra-axillary bolls of Pima cotton, showing difference in type of boll stems. The axillary bolls usually have long stems and often show some semblance of a joint

Not infrequently it may be observed that two cycles of flowering are in progress on the same fruiting branches, some of the later nodes bearing extra-axillary flowers while the older inner nodes may carry flowers on short axillary branches. (Fig. 6.) While under Arizona conditions a large number of these tardy floral buds may reach the flowering stage, it is only seldom that they reach maturity, on account of injury by frosts, and consequently they fail to play any important part in the final yield. However, where the seasons are longer, as in the valleys of southern California, it has been observed that in some seasons late-matured bolls from short axillary branches contribute materially to the yield.

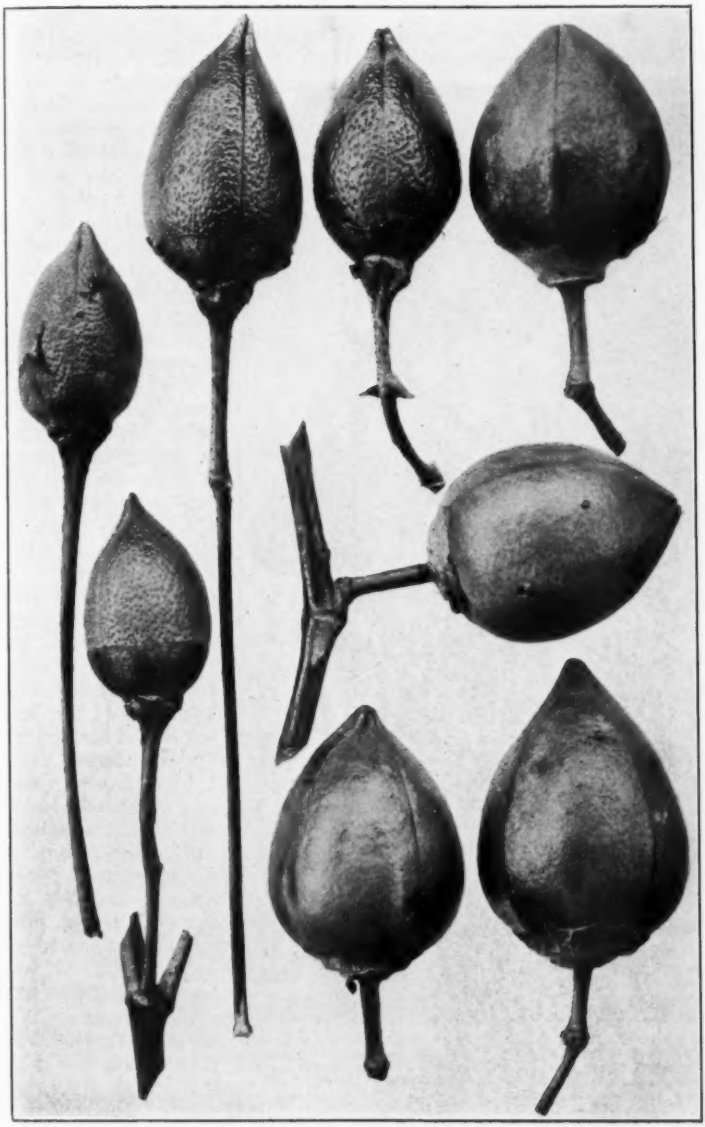

FIGURE 4.—Axillary bolls of Pima and Acala cotton, illustrating different types of pedicels. The four Pima bolls shown at the left and top center are smaller and have longer stems and are more deeply pitted than the four Acala bolls

EFFECT OF REMOVAL OF EXTRA-AXILLARY BUDS ON DEVELOPMENT OF AXILLARY FRUIT

Experiments were begun in 1926 to determine to what extent the retention and development of axillary buds could be influenced by the removal of the fruit buds in the extra-axillary positions. In this season the defruiting process was not started until June 17, after the plants had already developed several fruiting branches, some of which had borne buds that had almost reached the flowering stage.

FIGURE 5.—A fruiting branch of Acala cotton (in two sections), showing an axillary boll developing at each node late in the season. The extra-axillary fruit had shed naturally from the first four nodes, and the scars are clearly shown. The flower at the fifth node is from an extra-axillary bud

Twenty-seven normal Pima plants were selected from a group of well-grown plants in a single row about 200 feet long, and all the fruit buds were removed from 17 of them on that date. Ten other plants of about equal size, interspersed among these 17, were selected as controls and tagged for observation as to the development of axillary fruit under normal conditions. In an adjacent row planted to the Acala variety 10 plants were selected on the same day and 5 of them were defruited, the other 5 being designated as controls. From June 17 until September 1 the extra-axillary fruit buds on the 17 defruited Pima plants and the 5 defruited Acala plants were pinched out every two or three days and a tag was attached to the internode to show the date of removal. On October 11, diagrams were made of all of the plants to show the location of the axillary fruit and the number

of vacant nodes. The data obtained from these diagrams are shown in Tables 1 and 2.

In 1928 a more careful study of axillary fruit production was conducted on 10 Pima and 9 Acala plants from which the extra-axillary

FIGURE 6.—Fruiting branches of Acala cotton, showing axillary buds ready to flower, on nodes behind flowers and bolls that have been developed in the extra-axillary positions. Thus it sometimes happens on upland cotton that a second wave of flowering may be in progress after that which normally occurs

buds were removed daily and on 10 control plants of each variety which were allowed to grow normally. The defruiting process was started on June 1 and discontinued on September 4. The influence of this defruiting on the plants is shown by the data in Tables 3 and 4.

TABLE 1.—*Growth of main stem, number of nodes and axillary buds developed, axillary buds shed and percentage retained on Pima and Acala cotton plants on which the extra-axillary buds were removed every two or three days during the season, at the United States Field Station, Sacaton, Ariz., in 1926*

Variety and plant No.	Height of plant	Nodes on fruiting branches	Axillary buds recorded on fruiting branches June 17 to Sept. 1	Axillary buds recorded as shed before flowering	Axillary buds retained to Oct. 11	Axillary bolls matured on main stem	Extra-axillary buds removed during season
Pima:	*Inches*	*Number*	*Number*	*Number*	*Per cent*	*Number*	*Number*
1	72	169	64	16	75.0	12	116
2	70	120	51	25	51.0	9	99
3	73	118	44	25	43.2	4	95
4	68	84	31	12	61.3	4	71
5	73	138	52	21	59.6	4	131
6	78	146	55	27	50.8	10	118
7	69	102	43	22	48.8	5	91
8	66	68	28	10	64.3	7	58
9	73	99	39	15	61.5	5	88
10	80	126	44	8	81.8	6	101
11	72	94	33	17	48.5	2	74
12	81	264	83	20	61.2	17	176
13	84	197	124	55	55.7	15	147
14	74	187	106	46	56.6	15	138
15	79	133	77	40	48.1	11	109
16	78	190	85	43	49.4	10	152
17	80	163	87	38	56.3	10	132
Mean	74.7±0.85	141.1±8.25	61.5±4.60	25.9±2.43	57.24±1.68	8.6±0.74	111.52±5.26
Acala:							
1	75	265	146	66	54.8	5	185
2	65	267	163	78	52.2	23	197
3	61	265	141	84	40.4	8	171
4	69	216	108	66	38.9	3	166
5	68	225	127	84	33.9	4	170
Mean	67.6±1.65	247.6±8.01	137.0±6.64	75.6±2.92	44.04±2.89	8.6±2.65	177.8±4.14

TABLE 2.—*Growth of main stem and number of nodes and axillary and extra-axillary bolls produced on normally developed Pima and Acala cotton plants at the United States Field Station, Sacaton, Ariz., in 1926* [a]

Variety and plant No.	Height of plant	Nodes on fruiting branches	Axillary bolls matured on fruiting branches	Axillary bolls matured on main stem	Extra-axillary bolls matured
Pima:	*Inches*	*Number*	*Number*	*Number*	*Number*
1	66	79	0	0	47
2	64	108	4	0	73
3	65	118	1	0	85
4	60	49	0	1	40
5	66	64	0	0	44
6	61	70	0	0	52
7	51	50	0	0	33
8	65	65	1	0	39
9	58	43	0	0	27
10	58	56	1	1	38
Mean	61.4±1.03	70.2±5.50	0.7±0.213	0.2±0.09	47.8±3.96
Acala:					
1	56	102	0	0	21
2	59	120	0	0	35
3	65	114	0	0	25
4	59	99	0	0	28
5	64	130	0	0	36
Mean	60.6±1.21	113.0±4.11	0	0	29.0±2.07

[a] The total number of axillary buds which developed and shed on the normally developed plants was not recorded in 1926.

TABLE 3.—*Number of nodes, axillary buds developed, axillary buds shed, and percentage retained on Pima and Acala cotton plants on which the extra-axillary buds were removed daily during the season, at the United States Field Station, Sacaton, Ariz., in 1928* [a]

Variety and plant No.	Nodes on fruiting branches	Axillary buds recorded on fruiting branches June 1 to Sept. 4	Axillary buds recorded as shed before flowering	Axillary buds retained to flowering	Extra-axillary buds removed during season
Pima:	*Number*	*Number*	*Number*	*Per cent*	*Number*
1	214	150	120	20.0	175
2	184	125	99	20.8	160
3	173	127	106	16.5	158
4	176	120	93	22.5	155
5	162	107	96	10.3	144
6	155	90	81	10.0	136
7	176	118	81	31.4	158
8	150	87	74	14.9	133
9	189	117	93	20.5	172
10	199	136	115	15.4	169
Mean	177.8±4.32	117.7±4.23	95.8±3.27	18.23±1.39	156.0±2.93
Acala:					
1	143	90	63	30.0	114
2	130	54	31	42.6	113
3	255	161	97	39.8	205
4	116	54	39	27.8	92
5	99	47	31	34.0	74
6	137	51	34	33.3	92
7	173	64	28	56.3	115
8	119	33	4	87.9	78
9	127	39	17	56.4	87
Mean	144.3±10.73	65.9±9.09	38.2±6.20	45.34±4.22	107.8±9.18

[a] The height of plants and the number of axillary bolls matured on main stem were not recorded in 1928.

TABLE 4.—*Number of nodes, axillary buds developed, axillary buds shed, percentage retained to flowering, and number of extra-axillary buds retained, on normally developed Pima and Acala cotton plants at the United States Field Station, Sacaton, Ariz., in 1928* [a]

Variety and plant No.	Nodes on fruiting branches	Axillary buds recorded on fruiting branches June 1 to Sept. 4	Axillary buds recorded as shed before flowering	Axillary buds retained to flowering	Extra-axillary buds retained to flowering
Pima:	*Number*	*Number*	*Number*	*Per cent*	*Number*
1	79	47	44	6.4	20
2	80	53	51	3.8	36
3	68	42	42	0	29
4	78	40	40	0	24
5	69	38	37	2.6	28
6	71	37	36	2.7	24
7	64	34	32	5.9	24
8	53	30	29	3.3	20
9	51	25	25	0	25
10	63	36	35	2.8	25
Mean	67.6±2.23	38.2±1.76	37.1±1.66	2.75±.503	25.5±1.02
Acala:					
1	98	2	2	0	50
2	78	5	5	0	38
3	93	0	0	0	29
4	109	22	22	0	31
5	90	4	4	0	36
6	81	1	1	0	34
7	87	5	4	20.0	37
8	104	2	2	0	54
9	98	1	1	0	47
10	96	6	6	0	41
Mean	93.4±2.13	4.8±1.40	4.7±1.39	2.0±1.39	39.7±1.81

[a] The height of plants and the number of axillary bolls shed after flowering were not recorded in 1928.

It will be observed from these data that the removal of the fruit buds in the extra-axillary positions caused the plants to grow taller than normally and to produce a much greater number of internodes. The fruiting branches of the defruited plants were much longer than those of the normal plants and a greater number of fruiting branches was developed. In 1926 the mean number of internodes produced on the defruited Pima plants was 141 ± 8.3, while the mean number on the normal Pima plants was 70 ± 5.5. Since the observations were not continued throughout the season, the number of axillary buds recorded in the tables does not represent the total number that developed, as many buds had appeared and shed before the observations were begun, and a larger number appeared after September 1. In 1926 an average of 111 ± 5.3 extra-axillary buds were removed from the Pima plants and 178 ± 4.1 from the Acala plants, and in 1928 an average of 156 ± 2.9 buds per plant were removed from the Pima plants and 108 ± 9.2 from the Acala plants. A large proportion of axillary buds were developed to maturity on some of the defruited plants; in some cases three or four bolls were matured on a single branch. (Fig. 7.) In 1926 the mean percentage retained until the end of the season was 57.2 ± 1.7 for Pima and 44.0 ± 2.9 for Acala. The effects of the defruiting on the retention of the axillary buds did not become apparent on the Pima until the process had been in progress for some time. Very few axillary bolls were developed on the

FIGURE 7.—Axillary bolls produced on a single fruiting branch of a Pima cotton plant on which no extra-axillary fruit was allowed to develop

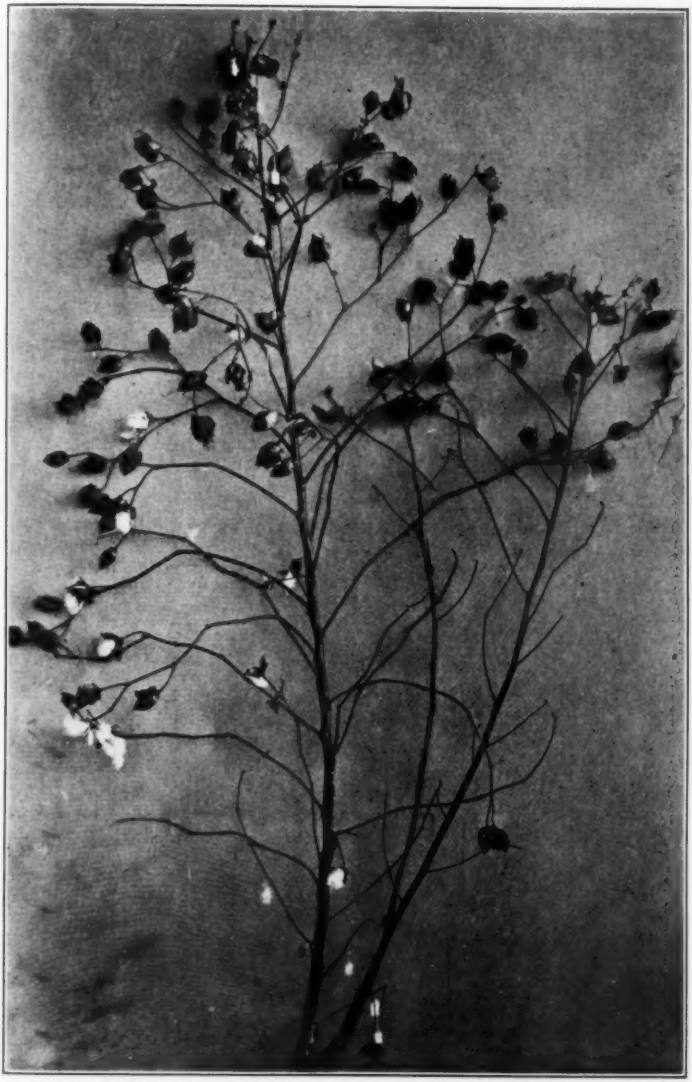

FIGURE 8.—Pima cotton plant showing bolls produced from axillary buds. The extra-axillary buds were removed every two or three days during the season

lower branches of the Pima plants, and at the end of the season most of the axillary bolls retained were found on the outer nodes of the middle branches and on the late-developed branches at the top.

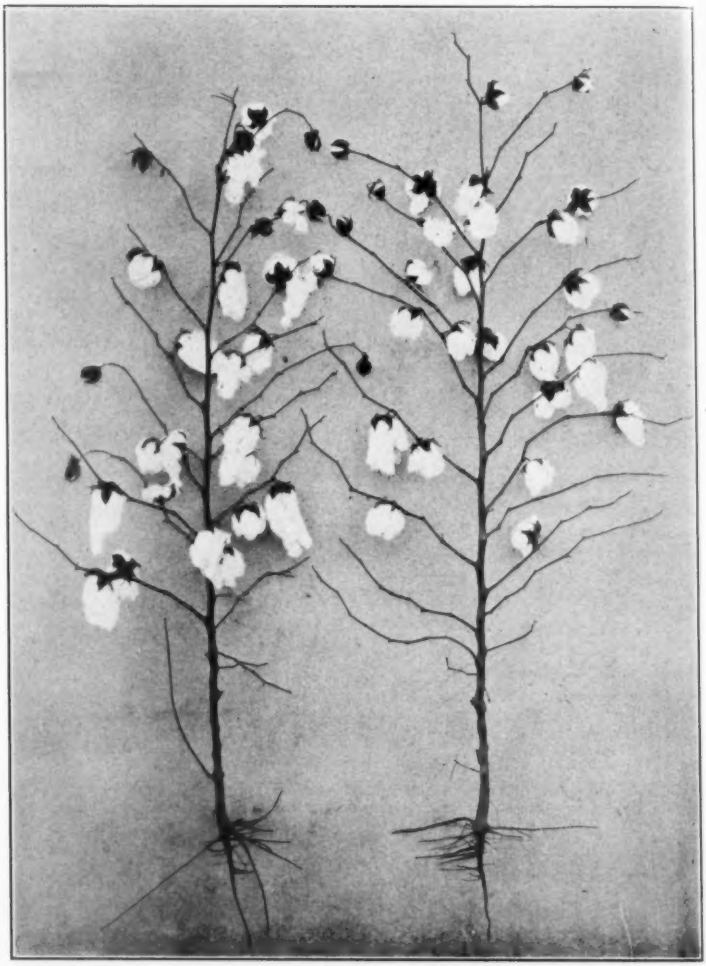

FIGURE 9.—Two Acala cotton plants that have produced bolls from axillary buds only. The extra-axillary buds were removed daily soon after they appeared. Note that many of these bolls are developed on nodes close to the main axis, while most of those on Pima cotton (fig. 8) developed on outer nodes and near the top of the plant. This is due to the fact that axillary buds on upland cotton usually remain dormant and may be stimulated to growth at any time, while on Pima cotton the axillary buds are shed or dry up on the plants a few days after appearance unless some stimulus occurs to force them into further development

(Fig. 8.) With the Acala variety, however, many of the axillary buds on the inner nodes of the lower branches became active after defruiting had been carried on for a time, and at the end of the season a considerable number of matured axillary bolls were found on the

lower and middle branches, even on the nodes near the main axis. (Fig. 9.)

It will be observed that very few axillary buds developed to maturity on the normal plants of Pima or Acala in either 1926 or 1928. However, plants grown under the most favorable conditions often develop to maturity a considerable number of axillary bolls in addition to the extra-axillary crop. In 1927 a count showed that the mean number of axillary bolls matured on five large, wide-spaced Pima plants was 6.4, in addition to a mean of 68.2 extra-axillary bolls.

In Arizona certain varieties show a greater tendency than others to produce axillary fruit. Plants of the sea-island type, which in this region usually do not produce any bolls until late in the season, often produce large numbers of axillary bolls on the upper fruiting branches and on the outer nodes of older branches. In 1927, 10 representative sea-island plants set an average of 9.3 axillary bolls per plant. The native southwestern varieties, Hopi and Sacaton Aboriginal, also show a tendency to produce an unusual amount of axillary fruit. Diagrams made of 10 Sacaton Aboriginal plants in 1927 showed an average of 10.2 axillary bolls per plant.

AXILLARY BUD DEVELOPMENT ON PLANTS AFFECTED WITH CRAZY-TOP DISORDER

In recent years there has appeared in many cotton fields of the Salt River Valley a peculiar disorder known as crazy top, which causes sterility and results in many abnormalities of the affected plants. In studying the effects of this disorder on the plants it has been observed that upon the arrival of cooler and more favorable weather conditions in the fall some of the more luxuriant upland plants that have been almost sterile from crazy top will begin to develp axillary branches and bear floral buds at many of the nodes from which extra-axillary bolls have been shed. Pima plants that have been rendered practically sterile by the disorder, although having no reserve axillary buds on the lower internodes of the branches as do the upland plants, are able to produce "supernumerary" bolls late in the season on new joints, by retaining a large proportion of the axillary buds. Frequently large clusters of bolls are observed in the tops of Pima plants affected with crazy top as a result of this tendency toward recovery. (Fig. 10.) As in the case of sterility from normal stress effects, the development of this fruit from axillary branches occurs late in the season, and it is not often that enough of it matures to be a factor in production. However, it is of interest from the standpoint of physiology and from its indication that there is a tendency toward recovery from the disease.

SHEDDING OF AXILLARY BUDS ON EGYPTIAN COTTON

It would appear that some investigators have failed to recognize that there is a difference in the morphology of the Egyptian and upland cotton plants with respect to the axillary buds. In studying the relative proportions of buds, flowers, and bolls from 1,300 Egyptian plants at Giza, in Egypt, Balls [5] collected weekly all the mate-

[5] BALLS, W. L. THE COTTON PLANT IN EGYPT. 202 p., illus. London. 1912.

rial beneath the plants and presented an analysis of the results in the form of shedding curves.

Bailey and Trought,[6] working with the Ashmouni, Assili, and Sakellaridis varieties, found "that the great majority of buds fall off at a very early stage when the bud is only about 2 mm. or less in width measured across the epicalyx." In an experiment carried out in 1923 they reported that the number of buds and bolls which were shed by 40 Sakellaridis plants during the period June 26 to September 26 was as follows:

Small buds (not more than 5 mm. in diameter)	787
Larger buds	275
Bolls (all ages)	92

From these data they concluded that "about 60 per cent of the 'possible crop' was lost in this case through bud shedding, while

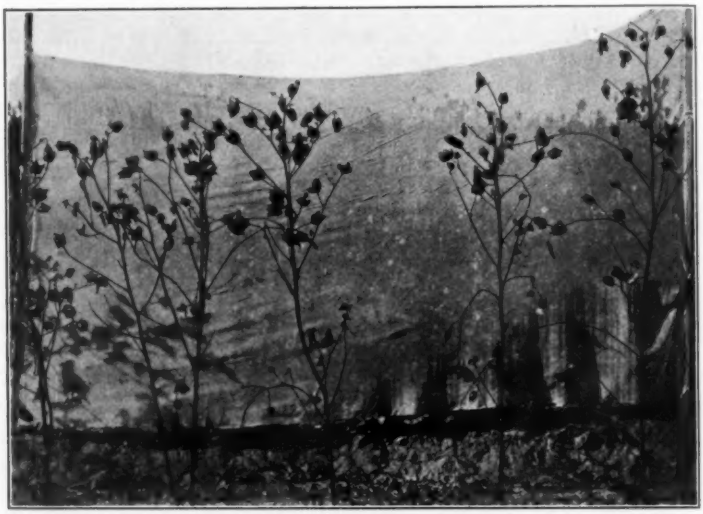

FIGURE 10.—Pima cotton plants as they commonly appear late in the season when affected by the crazy-top disorder. It will be noted that most of the bolls that are set are on the terminal nodes. A part of the crazy-clustered appearance of the bolls is due to the retention of the axillary fruits

boll shedding accounted for about 6 per cent of the total." In this experiment, as in those conducted in 1921 and 1922, these investigators used the "catch-net" system of collecting all of the buds that dropped off each day and classified them into ages according to size.

The experiments conducted by the writer indicate that in a study of bud shedding the method of collecting the fallen buds from the ground or in catch nets is open to serious error. Should this method be followed with Pima Egyptian cotton, the number of squares falling into the class measuring less than 5 mm. across the epicalyx would be far too high if considered as an actual loss of "possible crop," since it is never possible to obtain two bolls at each node throughout the plant, no matter how favorable the conditions.

[6] BAILEY, M. A., and TROUGHT, T. GROWTH, BUD-SHEDDING, AND FLOWER PRODUCTION IN EGYPTIAN COTTON. Egypt Min. Agr., Tech. and Sci. Bul. 65, 8 pp., illus. 1927.

Obviously, many of the rudimentary axillary buds, which emerge with as great regularity as the "normal" buds, but which only rarely develop into bolls, would be included in the count, since under ordinary conditions they drop off when at a size of from 1 to 5 mm. across the epicalyx, though many shrivel up and remain attached to the plants for long periods.

The great similarity of these axillary buds and the extra-axillary buds would make it impracticable to distinguish between the two kinds if all were collected together. (Figs. 11 and 12.) It will be seen from Table 4 that the mean number of axillary squares developed on the normal Pima plants between June 1 and September 4, 1928, was 38 ± 1.8. Practically all of these were shed, and had they been counted with the shed extra-axillary squares the total number would have been nearly twice as great as the mean number of vacant extra-axillary nodes on the plants.

SUMMARY

The axillary buds and branches on the fertile branches of Egyptian cotton differ in morphology and behavior from those on upland cotton.

In the Pima variety of Egyptian cotton rudimentary axillary branches begin development with the advent of minute triangular buds in the axil of each leaf, but ordinarily this development is cut short by the shedding or drying up of the buds, which maintain active growth for only a few days.

In upland varieties the axillary buds usually remain dormant, but they can be stimulated to growth at any time later in the season.

In Arizona, axillary buds on upland plants frequently begin development late in the season, especially on luxuriant plants that have shed excessively during the summer, and a second cycle of flowering may occur on the older fruiting branches.

The axillary buds and bolls on both types of cotton are usually developed too late to contribute materially to the yield under Arizona conditions.

Removal of the extra-axillary buds artificially by pinching off the new buds at frequent intervals resulted in more axillary buds being retained by the Pima plants and many of them continued development to maturity.

The removal of the extra-axillary buds on upland plants stimulated many of the axillary buds into development, and a considerable number of them were retained by the plants until maturity.

On the defruited Pima plants the axillary fruits that matured were located only on the internodes developed late in the season, while on the defruited upland plants a greater part of them matured on the internodes developed earlier in the season.

The artificial removal of the extra-axillary buds caused the plants to grow much taller and to produce longer and more numerous fruiting branches than normally.

Both Pima and upland plants that have been rendered almost sterile during the summer from effects of the crazy-top disorder may develop a large number of axillary fruits late in the season, those on

FIGURE 11.—Axillary buds from Pima cotton plants, collected when ready to become separated from the plants by abscission. Note that some are almost identical in shape with the extra-axillary squares shown in Figure 12. × 10

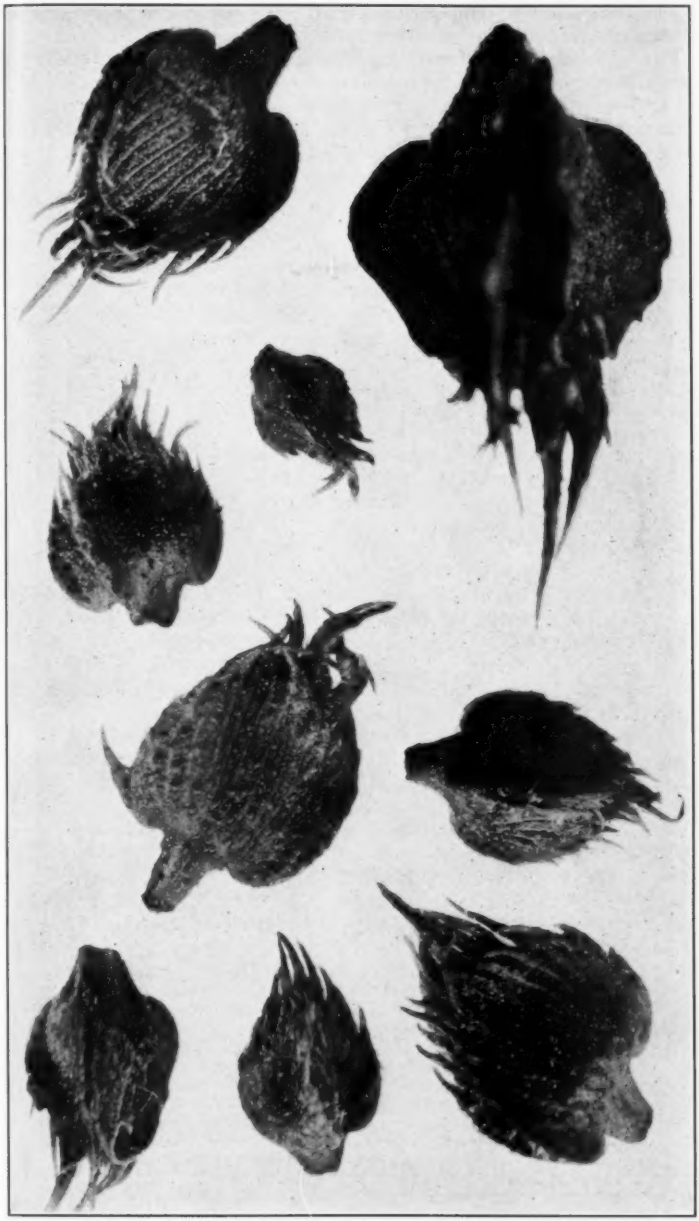

FIGURE 12.—Extra-axillary squares from Pima cotton plants, collected when only a few days old. × 10

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the upland plants being produced on both early and late developed internodes, while those of Pima form only on the late growth.

The large numbers of very small buds that are shed naturally from the axillary positions on Egyptian cotton make it impracticable to use the method of collecting the shed squares or young bolls as a basis for estimating the "possible crop" or for assigning a proper ratio of shed buds and bolls, as has been attempted by some investigators.

LINUM NEOMEXICANUM (YELLOW PINE FLAX) AND ONE OF ITS POISONOUS CONSTITUENTS [1]

By W. W. Eggleston, *Assistant Botanist*, O. F. Black, *Senior Biochemist*, and J. W. Kelly, *Junior Biochemist, Office of Drug and Related Plants, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The senior writer, while examining plants on a range where cattle had been poisoned about Willow Spring tank, on the western slope of Sitgreaves Mountain, Tusayan National Forest, Ariz., noticed small patches of yellow pine flax (*Linum neomexicanum*). A few days later, in the Tonto National Forest, this same species was found in abundance along the old wagon road from Pine, Ariz., to Flagstaff, near the top of Mogollon Rim. This wagon road was in use for many years as a stock trail and for freighting purposes, but is now paralleled by an automobile road. The place where the flax grew in greatest abundance was the site of an old camp ground, and much stock had been poisoned in that vicinity. Although there was no direct evidence to connect *L. neomexicanum* with these fatalities, samples were collected and sent to the laboratory, where they were examined, with the result that a poison of glucosidal nature was isolated. The object of the present paper is to give a botanical description of the plant and some facts concerning the poison derived from it.

In the fall of 1880 E. L. Greene discovered this interesting species while living at a little mining camp of Pinos Altos in the Pinos Altos Mountains, Grant County, N. Mex., and described it in the Botanical Gazette of March, 1881.[2]

The plant is now known in New Mexico, north to the middle and west forks of Gila River, east to the Black Range, and south in the Big Burro and San Luis Mountains to Guayanopa Canyon in the Sierra Madre of Chihuahua. In the southeastern Arizona it is known in the Chiricahua Mountains, the Huachuca, the Rincon, the Santa Rita, the Pinal and the Mule Mountains, and extends northwestward along Mogollon Rim to Sitgreaves and Cedar Mountains.

Two other wild flaxes are found in the grazing country, and both of them are suspected of poisoning sheep. One, *Linum lewisii*, a blue-flowered flax, occurs on the higher mountain ranges; the other, *L. rigidum*, a yellow-flowered species, occurs in the Pecos Valley in Texas.

BOTANICAL DESCRIPTION

Linum neomexicanum Greene. Yellow pine flax. (Fig. 1.) Annual, or winter annual herb, flowering in August, erect, glabrous, slightly glaucous, stems simple, branched from near the base, slender, angled, 1 to 2 feet tall; lower leaves opposite below, becoming alternate above, erect or ascending, sessile, glabrous, about one-half inch long, lanceolate, oblong, entire, acute, crowded near the base, becoming narrower and farther apart above, bracts lanceolate to linear; pedicels one-fourth to one-half inch long; sepals 5, persistent, lanceolate to ovate, acumi-

[1] Received for publication July 15, 1930; issued November, 1930.

[2] Greene, E. L. NEW PLANTS OF NEW MEXICO AND ARIZONA. Bot. Gaz. 6: 183–185. 1881.

FIGURE 1.—*Linum neomexicanum* (yellow pine flax)

nate, glandular, serrate or entire, about one-fourth inch long, shorter than the carpels; petals 5, yellow, one-fourth inch long; stamens 5, longer than the sepals, united at the base; filaments linear-lanceolate; capsules ovoid, incompletely 10-celled, one-fourth inch wide, unribbed; seeds flattened, oblong in outline, light brown, shining.

Distribution.—New Mexico, Arizona, and Chihuahua (Mexico).

Altitude.—5,000 to 8,000 feet.

TOXICITY

No member of the family Linaceae has hitherto been proved to contain material that is actively poisonous, although it is well known that some of them possess the cyanogenetic glucoside linarmarin. Glucosides of this type, however, are found in other plants commonly used for forage, as, for instance, sorghum, and in feedstuffs, as flaxseed cake, which is frequently used as a concentrate for fattening cattle, although it usually contains small amounts of hydrocyanic acid. *Linum catharticus*, so far as the writers have been able to discover, is the only species of flax known to possess physiologically active properties, and even in this case it is a matter of dispute whether the substance found, linin, has the purgative effects that were earlier attributed to it.[3]

EXPERIMENTAL WORK

The air-dried plant was ground to moderate fineness and 50 gm. was tested for the presence of hydrocyanic acid by distillation with acid, and the distillate was treated for Prussian blue. The results, however, were negative. The same procedure was repeated after the ground material had been allowed to macerate for an hour in the presence of emulsin. This also yielded a negative result. These tests, of course, do not show conclusively that the fresh plant does not contain hydrocyanic acid, but the probabilities are that it does not.

As a preliminary test for toxicity, an aqueous extract was prepared by steeping 60 gm. of the plant in successive portions of hot water until the material was thoroughly exhausted of water-soluble substances. The water extract was evaporated on the steam bath to small bulk, filtered, and made up to exactly 100 c. c. One-half of this, representing 30 gm. of the plant, was fed to a rabbit per os by means of a stomach tube. The animal seemed little affected, although it was inert and dejected during the day, but that night it died. Next, a white mouse was given subcutaneously one-fourth c. c. of the same solution, equivalent to 0.15 gm. of the plant, and manifested the same behavior, dying within 18 hours. The results indicated that the plant contains a poisonous substance or substances.

To gain a clearer idea of the nature of this poisonous material, 180 gm. of the ground plant was extracted in a Sohxlet apparatus, first with ether, then with alcohol, and finally with water. All three of these extracts, when given subcutaneously to mice in very dilute water solution, proved fatal, and the animals exhibited the same symptoms in every case. The ether extract, on evaporating off the solvent, taking up the water, and evaporating to dryness, left a minute quantity of light-yellow gum only slightly soluble in water, from which it separated in concentrated solution in the form of yellow oily globules. A similar material was prepared from the alcohol and water extracts, much the greater quantity being found in alcohol.

[3] HILLS, J. S., and WYNNE, W. P. LININ. [London] Chem. Soc. Proc. 21: 74. 1905.
KOBERT, R. DIE ABFÜHRENDE WIRKUNG VON LINUM CATHARTICUM. Pharm. Ztg. 50: 370. 1905.

It seemed to follow from these results—namely, that the poisoned animals exhibited the same symptoms from each of the extracts and the same material could be isolated from all three extracts—that a single poisonous substance was involved which was sparingly soluble in water and in ether, but much more so in alcohol.

PREPARATION OF THE POISON

The dried ground plant was thoroughly extracted with boiling 95 per cent alcohol. The alcohol was distilled from the extract, the residue taken up with water, and the precipitated chlorophyll filtered off. Lead acetate was added to the solution, the resulting precipitate removed by filtration, and the excess of lead with hydrogen sulphide. The aqueous solution thus obtained was concentrated to small bulk on the water bath and then shaken out several times with acetic ether, which removed the active principle. On evaporating the solvent, a light-yellow gummy residue remained, which gradually dried to a hard, brittle, resinous material. By this procedure it was found that 100 gm. of the plant yielded 6.6 gm. of the crude poison, or 6.6 per cent. That the material prepared as described contains the active principle in the plant was proved beyond any doubt by its effect on mice. Given by injection or eaten on bread it proved fatal in every case, with the symptoms as described above.

CHEMICAL PROPERTIES OF THE POISON

The toxic substance is amorphous and has so far resisted efforts to crystallize it. It contains no nitrogen. It reduces Fehling's solution after boiling with dilute hydrochloric acid, but not before; and this property in conjunction with its solubility in acetic ether, suggests the probability that it is a glucoside. No recognizable products resulting from its hydrolysis were found. Likewise, no significant substances were found by either oxidizing or reducing agents. With concentrated nitric acid it gives a brilliant carmine red color which gradually fades to brown. It yields a permanent foam when shaken with water, suggesting a saponin. It dissolves in water to the extent of 3 parts to 100 at 20° C., and is more soluble in hot water. It is very soluble in ethyl or methyl alcohol, but sparingly soluble in most of the other common organic solvents.

In what has preceded the writers have endeavored to show that *Linum neomexicanum* contains a poison of a highly toxic nature, and that it should be classed among plants dangerous to animals. The poison is probably a glucoside and slow in action but fatal in minute doses when administered either subcutaneously or per os. The new substance has been provisionally named "linotoxin."

PHOTOPERIODIC RESPONSE OF SOYBEANS IN RELATION TO TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS[1]

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INTRODUCTION

In earlier papers[2] [3] dealing with the effect of relative length of day and night on plant growth and development, considerable attention was given to the contrasted responses of early and late varieties of soybeans to changes in day length. These contrasted responses seemed to be correlated with the changes in behavior of the different varieties under field conditions with advance of the season. Of the four varieties studied under field conditions in the vicinity of Washington, D. C., the Biloxi consistently behaved as a very late variety, earliest plantings first coming into flower about September 1 and failing to mature seed; the Tokyo was somewhat earlier, the first plantings usually flowering early in August and successfully maturing seed; the Peking flowered about 10 days earlier than the Tokyo, or toward the close of July, being well adapted to the region; the Mandarin behaved as an early variety, flowering 3 to 4 weeks after germination. Thus the normal vegetative periods of early plantings of the four varieties, in the order named, are about 95, 65, 55, and 25 days, respectively.

It was found that, when early plantings of the four varieties are exposed to an artificially shortened daylight period of 10 to 12 hours or less, all varieties tend to flower at about the same time, namely, 20 to 25 days after germination. In other words, all behave as early varieties. Again, in field plantings of the four varieties made at intervals of three or four days through a single growing season, the vegetative period of plantings of the Mandarin made during the months of June and July did not change materially; while in the other varieties there was a progressive shortening of the vegetative period as the season advanced. The latest variety, Biloxi, showed the maximum shortening of the preflowering period of growth with advance of the season. Hence, in the late plantings there is, again, an evident tendency in all varieties to behave as when exposed to an artificially shortened day length in early summer. However, the duration of the vegetative period of the late plantings failed to reach the lower level of 20 to 25 days obtained with the shortened day lengths in early summer. Moreover, a considerable lengthening of the vegetative period was observed in the very early plantings of all

[1] Received for publication June 28, 1930; issued November, 1930.

[2] GARNER, W. W. and ALLARD, H. A. EFFECT OF RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

[3] ——— and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

varieties, including the Mandarin. The tentative conclusion was reached that under practical conditions in the field the differences in behavior of soybean varieties with respect to time of flowering are due primarily to length of day, while the relatively low temperatures of late spring and early fall exercise a retarding influence on the flowering stage in both the very early and the very late plantings of all varieties.

METHODS

It seemed probable that the relationships existing between the early and late forms of soybeans were more or less applicable to a large group of species of which the different forms normally flower on different dates in early and late summer and early fall. It appeared very desirable, therefore, to obtain more definite data on the interrelationship of length of day and temperature as influencing the initiation of reproductive activity in the different varieties of soybeans under natural conditions in the field. Such information should be of considerable value in arriving at a conclusion as to the actual significance of length of day as a factor in the natural distribution of plants and their adaptation to different latitudes.

For this purpose three series of experiments were carried out. In the first series, field plantings of the four varieties of soybeans were made at regular intervals of four or five days throughout the growing season at Washington for the years 1920 and 1922 to 1927, inclusive. The results in this series furnish a basis for determining the normal behavior of the different varieties with the advance of the open season in the vicinity of Washington. In the second series of tests, plantings were made in the greenhouse at regular intervals throughout the year and the mean temperature was maintained at an approximately uniform level. In the third series, beginning as early in the spring as outside conditions would permit, plantings of the soybeans were made in boxes in the greenhouse at regular intervals, and as soon as germination had taken place the plants were subjected to a fixed day length of 10 hours under approximately outdoor conditions. To accomplish this the usual arrangement of trucks on steel tracks and ventilated dark houses was employed.[4] The plants in the first series, of course, were subjected to the natural conditions of day length and temperature prevailing during the open growing season; those in the second series were exposed to the natural change in day length taking place throughout the year, but under a relatively fixed mean temperature approximating that of midsummer; the only essential difference between the treatment given in the third series and that given in the first series was that the plants in the third series were exposed to a constant day length of short duration.

EXPERIMENTAL DATA

FIELD PLANTINGS MADE AT INTERVALS THROUGH THE GROWING SEASON

The plantings were made at intervals of three to five days through the season in accordance with the plan followed in 1919, as described in the writers' first paper.[5] The tests were carried out each year from

[4] A description of the outfit used is contained in an earlier publication. See GARNER, W. W., and ALLARD, H. A. Op. cit., p. 559. (See footnote 2.)

[5] GARNER, W. W., and ALLARD, H. A. Op. cit., p. 569. (See footnote 2.)

1920 to 1927, inclusive, except in 1921. The period required for germination of the seed naturally varied somewhat, depending on prevailing weather conditions, and consequently the dates of germination did not conform rigidly to the schedule of plantings. In order to tabulate the experimental data in summarized form, the actual dates of germination were arbitrarily grouped into periods of one week each and the middle of the week was taken as the average date of germination for a group. For example, all plantings germinating during the week of May. 16 to 22 are classed as having germinated on May 19. In some instances an error of one or two days for the averaged dates of germination may be involved, but the results are sufficiently accurate for practical purposes. The earliest date on which flowering had become general in each planting was noted, as was the average height attained by the plants. The results are presented in summarized form in Tables 1, 2, 3, and 4. The data previously obtained in 1919 also are included in the tables. Because of poor seed the Tokyo plantings in 1923 were largely a failure and are not included in the tables.

The average values for the 8-year period are shown graphically in Figure 1. The graph for Biloxi plantings in the greenhouse covering the same period of the year, as described under the next section, is added to facilitate comparison. The seasonal change in length of day, which of course is essentially uniform from year to year, is shown graphically in Figure 2. The average mean temperature during the vegetative period of each planting of the four varieties for the eight years was computed from the Weather Bureau records, and the values applicable to the Mandarin and Peking varieties are shown in Figure 1. The data for Tokyo and Biloxi are very nearly the same as those for Mandarin and Peking and hence are omitted. Naturally, the plantings of late June and early July experienced the highest average temperature (75° to 77° F.) for the preflowering period of growth. In considering these temperature data, as shown in Figure 1, it is to be remembered that the figures do not refer to specific calendar periods indicated in the chart but rather to the periods of time elapsing between germination and first flowering in the particular plantings indicated in each case.

The yearly fluctuations in time of flowering of plantings germinating on any given date are considerable, the extreme range being about 15 days. Also, the heights of the plants vary widely from year to year. Presumably length of day is not a significant factor in these yearly fluctuations, but irregularities in temperature and other variable factors might well account for the observed fluctuations. To afford ready comparison of the variations in length of the vegetative period of the soybeans and differences in the prevailing temperature, the detailed data for the Biloxi and Mandarin varieties, together with the mean temperatures of the vegetative periods in 1925 and 1927, are shown in Figure 3.

TABLE 1.—*Dates of germination and flowering, duration of vegetative period, and average heights of the Mandarin variety of soybeans planted at intervals of three to five days through the open growing season at the Arlington Experiment Farm, Rosslyn, Va., in the years 1919, 1920, and 1922–1927*

Average date of germination	Date of first blossoming in—									Duration of vegetative period in—									Average height of plants in—							
	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average
										Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
May 19	June 16	June 25	June 17	June 17	.....	June 16	June 26	June 26	June 20	28	37	29	29	.....	28	38	38	32	...	19	...	16	...	11	8	11	13
May 26	June 22	June 27	June 21	June 13	June 28	June 22	June 29	June 27	June 25	27	32	26	28	33	27	34	32	30	...	19	...	17	13	12	8	11	13
June 2	June 28	June 30	June 25	June 25	July 3	June 27	July 3	July 2	June 29	26	28	23	23	31	25	31	30	27	...	20	...	18	13	12	8	12	14
June 9	July 6	July 5	July 2	July 4	July 8	July 1	July 9	July 8	July 5	27	26	23	25	29	22	30	29	26	...	21	...	18	15	12	9	11	14
June 16	July 13	July 11	July 11	July 9	July 12	July 8	July 14	July 14	July 12	27	25	25	23	26	22	28	28	26	...	21	...	17	14	11	9	11	14
June 23	July 21	July 18	July 17	July 18	July 18	July 20	July 21	July 18	July 19	28	25	24	25	25	26	28	25	26	...	21	...	17	14	13	10	13	15
June 30	July 28	July 24	July 23	July 23	July 24	July 23	July 25	July 24	July 24	26	24	23	23	24	23	25	24	24	...	17	...	18	14	14	11	10	14
July 7	July 31	July 31	July 30	Aug. 1	July 30	July 29	July 30	July 30	July 30	24	24	23	25	23	22	23	23	23	...	17	...	20	14	15	10	9	14
July 14	Aug. 7	Aug. 7	Aug. 5	Aug. 8	Aug. 7	Aug. 6	Aug. 5	Aug. 6	Aug. 6	24	24	22	25	24	23	22	23	23	...	17	...	20	17	18	10	11	16
July 21	Aug. 15	Aug. 16	Aug. 14	Aug. 12	Aug. 13	Aug. 14	Aug. 10	Aug. 14	Aug. 13	25	26	24	22	23	24	20	24	24	...	16	...	19	17	15	7	11	14
July 28	Aug. 21	Aug. 17	Aug. 21	Aug. 23	Aug. 21	Aug. 20	Aug. 17	Aug. 27	Aug. 21	24	30	24	27	24	23	20	29	24	...	20	...	17	16	10	7	10	13
Aug. 4	Aug. 28	Aug. 25	Aug. 30	Aug. 29	Aug. 26	Aug. 27	Aug. 28	Sept. 4	Aug. 29	24	21	26	25	22	24	24	31	25	...	19	...	15	15	9	9	9	13
Aug. 11	Sept. 7	Sept. 4	Sept. 5	Sept. 4	Sept. 3	Sept. 5	Sept. 4	Sept. 14	Sept. 6	27	24	25	24	23	25	24	31	25	...	17	...	15	12	13	9	9	13
Aug. 18	Sept. 11	Sept. 13	Sept. 11	Sept. 17	Sept. 16	Sept. 9	Sept. 12	Sept. 16	Sept. 13	24	26	24	30	29	22	25	28	26	...	16	...	16	10	12	9	7	12
Aug. 25	.....	Sept. 25	Sept. 19	Sept. 25	Sept. 20	Sept. 16	.....	Sept. 22	Sept. 21	.....	31	25	31	26	22	...	30	28	...	12	...	15	9	11	...	7	11
Sept. 1	.....	.....	Sept. 27	Oct. 1	.....	Sept. 23	Sept. 23	Oct. 4	Sept. 28	.....	.....	26	30	.....	22	22	33	27	...	...	...	14	...	9	8	7	10

TABLE 2.—*Dates of germination and flowering, duration of vegetative period, and average heights of the Peking variety of soybeans planted at intervals of three to five days during the open growing season at the Arlington Experiment Farm, Rosslyn, Va., in the years 1919, 1920, and 1922–1927*

Average date of germination	Date of first blossoming in—									Duration of vegetative period in—									Average height of plants in—								
	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average
										Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
May 19	July 12	July 29	July 18	July 16	.....	July 13	July 28	July 30	July 21	54	71	60	58	.....	55	70	72	63	...	31	...	30	...	25	14	20	24
May 26	July 18	July 30	July 19	July 19	July 31	July 18	...do...	...do...	July 24	53	65	54	54	66	53	63	65	59	...	30	...	30	25	27	14	20	24
June 2	July 29	Aug. 1	July 23	July 24	Aug. 1	July 23	...do...	...do...	July 28	57	60	51	52	60	51	56	58	56	...	32	...	32	28	26	15	22	26
June 9	Aug. 4	Aug. 3	July 29	July 27	Aug. 4	Aug. 1	Aug. 2	Aug. 1	Aug. 1	56	55	50	48	56	53	54	53	53	...	36	...	33	25	21	12	20	25
June 16	Aug. 7	Aug. 5	Aug. 2	Aug. 3	Aug. 7	Aug. 2	Aug. 4	Aug. 5	Aug. 4	52	50	47	48	52	47	49	50	49	...	38	...	32	28	22	15	17	25
June 23	Aug. 11	Aug. 10	Aug. 6	Aug. 10	Aug. 9	Aug. 11	Aug. 6	Aug. 9	Aug. 9	49	48	44	48	47	49	44	47	47	...	35	...	30	27	21	13	15	24
June 30	Aug. 15	Aug. 13	Aug. 10	Aug. 14	Aug. 14	Aug. 13	Aug. 10	Aug. 10	Aug. 12	46	44	41	45	45	44	41	41	43	...	33	...	30	25	23	11	15	23
July 7	Aug. 18	Aug. 18	Aug. 15	Aug. 19	Aug. 20	Aug. 18	Aug. 13	Aug. 15	Aug. 17	42	42	39	43	44	42	37	39	41	...	31	...	27	25	22	11	13	22
July 14	Aug. 21	Aug. 21	Aug. 20	Aug. 25	Aug. 26	Aug. 23	Aug. 18	Aug. 20	Aug. 22	38	38	37	42	43	40	35	37	39	...	28	...	26	23	20	12	13	20
July 21	Aug. 25	Aug. 23	Aug. 26	Aug. 28	Aug. 30	Aug. 31	Aug. 20	Aug. 29	Aug. 27	35	33	36	38	40	41	30	39	37	...	27	...	26	23	17	11	11	19
July 28	Sept. 6	Aug. 31	Sept. 3	Aug. 31	Sept. 3	Sept. 3	Aug. 30	Sept. 3	Sept. 2	40	34	37	34	36	37	33	37	36	...	26	...	21	21	19	11	9	18
Aug. 4	Sept. 7	Sept. 2	Sept. 8	Sept. 6	Sept. 4	Sept. 5	Sept. 2	Sept. 13	Sept. 6	34	29	35	33	31	32	29	40	33	...	25	...	21	14	16	10	9	16
Aug. 11	Sept. 11	Sept. 9	Sept. 11	Sept. 10	Sept. 10	Sept. 8	Sept. 9	Sept. 19	Sept. 11	31	29	31	30	30	28	29	39	31	...	22	...	20	12	14	9	8	14
Aug. 18	Sept. 18	Sept. 18	Sept. 15	Sept. 21	Sept. 17	Sept. 13	Sept. 15	Sept. 28	Sept. 18	31	31	28	34	30	26	28	41	31	...	20	...	20	13	10	10	8	14
Aug. 25	Sept. 26	Sept. 25	Sept. 22	Sept. 28	Oct. 2	Sept. 20	.....	Oct. 4	Sept. 27	32	31	28	34	38	26	.....	40	33	...	17	...	16	11	8	.....	8	12
Sept. 1	.....	.....	Oct. 1	Oct. 1	.....	Sept. 26	Sept. 29	Oct. 5	Sept. 30	.....	.....	30	30	.....	25	28	34	29	...	...	...	17	...	6	9	7	10

TABLE 3.—*Dates of germination and flowering, duration of vegetative period, and average heights of the Tokyo variety of soybeans planted at intervals of three to five days during the open growing season at the Arlington Experiment Farm, Rosslyn, Va., in the years 1919, 1920, 1922, and 1924–1927*

Average date of germination	Date of first blossoming in—									Duration of vegetative period in—									Average height of plants in—								
	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average
										Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
May 19	July 29	Aug. 3	July 24	.....	.....	Aug. 3	Aug. 4	Aug. 8	Aug. 2	71	76	66	.....	.....	76	77	81	75	...	35	...	...	...	36	25	32	32
May 26	Aug. 1	...do...	...do...	.....	Aug. 11	Aug. 8	...do...	...do...	Aug. 4	67	69	59	.....	77	74	70	74	70	...	35	...	...	33	42	20	30	32
June 2	Aug. 11	Aug. 4	July 28	.....	...do...	Aug. 10	Aug. 7	...do...	Aug. 7	70	63	56	.....	70	69	66	67	66	...	35	...	...	33	42	23	28	32
June 9	Aug. 15	Aug. 9	Aug. 7	.....	Aug. 12	Aug. 13	Aug. 13	Aug. 15	Aug. 12	67	61	59	.....	64	65	65	67	64	...	40	...	...	33	36	20	28	31
June 16	Aug. 19	Aug. 11	Aug. 11	.....	Aug. 17	Aug. 16	Aug. 17	Aug. 16	Aug. 15	64	56	56	.....	62	61	62	61	60	...	42	...	...	35	29	20	27	31
June 23	Aug. 20	Aug. 16	Aug. 13	.....	Aug. 23	Aug. 22	Aug. 18	Aug. 18	Aug. 19	58	54	51	.....	61	60	58	58	57	...	40	...	...	34	27	18	22	28
June 30	Aug. 21	Aug. 19	Aug. 15	.....	Aug. 26	Aug. 25	Aug. 22	Aug. 23	Aug. 22	52	50	45	.....	57	56	53	54	53	...	37	...	...	32	28	18	20	27
July 7	Aug. 27	Aug. 22	Aug. 21	.....	Aug. 29	Aug. 30	Aug. 28	Aug. 30	Aug. 27	51	46	45	.....	53	54	52	54	51	...	36	...	...	29	24	20	17	25
July 14	Aug. 31	Aug. 29	Aug. 24	.....	Sept. 1	Sept. 2	Aug. 30	Sept. 4	Aug. 31	48	46	41	.....	49	50	47	52	48	...	33	...	...	25	29	20	16	25
July 21	Sept. 5	Aug. 31	Aug. 30	.....	Sept. 2	Sept. 7	Sept. 3	Sept. 8	Sept. 3	46	41	40	.....	43	48	44	49	44	...	32	...	...	25	27	20	15	24
July 28	Sept. 10	Sept. 3	Sept. 6	.....	Sept. 7	Sept. 8	...do...	Sept. 10	Sept. 7	44	37	40	.....	41	42	37	44	41	...	31	...	...	20	26	18	12	21
Aug. 4	Sept. 11	Sept. 5	Sept. 10	.....	Sept. 15	Sept. 11	Sept. 14	Sept. 17	Sept. 12	38	32	37	.....	42	38	41	44	39	...	26	...	...	17	22	19	12	19
Aug. 11	.....	Sept. 13	Sept. 13	.....	Sept. 24	Sept. 15	Sept. 22	Sept. 19	Sept. 18	.....	33	33	.....	44	35	42	39	38	...	23	...	...	16	20	19	12	18
Aug. 18	.....	Sept. 23	Sept. 20	.....	Oct. 3	Sept. 17	Sept. 25	Sept. 28	Sept. 24	.....	36	33	.....	46	30	38	41	37	...	21	...	...	14	15	17	12	19

TABLE 4.—*Dates of germination and flowering, duration of vegetative period, and average heights of the Biloxi variety of soybeans planted at intervals of three to five days during the open growing season at the Arlington Experiment Farm, Rosslyn, Va., in the years 1919, 1920, and 1922–1927*

Average date of germination	Date of first blossoming in—									Duration of vegetative period in—									Average height of plants in—								
	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average	1919	1920	1922	1923	1924	1925	1926	1927	Average
										Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
May 19	Sept. 4	Sept. 3	Sept. 4	Aug. 30	.....	Aug. 29	Sept. 1	Sept. 6	Sept. 2	108	107	108	103	.....	102	105	110	106	...	50	52	51	...	36	29	36	42
May 26	Sept. 5	...do...	...do...	...do...	Sept. 2	Sept. 3	...do...	...do...	Sept. 3	102	100	101	96	99	100	98	103	100	...	50	58	51	42	36	29	39	44
June 2	Sept. 7	Sept. 5	...do...	Sept. 2	...do...	...do...	Sept. 2	...do...	Sept. 4	97	95	94	92	92	93	92	96	94	...	51	53	50	43	36	35	40	44
June 9	Sept. 10	Sept. 7	Sept. 7	Sept. 4	Sept. 3	Sept. 6	Sept. 3	Sept. 7	Sept. 6	93	90	90	87	88	89	86	90	89	...	53	51	50	41	34	30	39	43
June 16	Sept. 13	Sept. 9	...do...	Sept. 9	Sept. 9	Sept. 9	...do...	Sept. 11	Sept. 9	89	85	83	85	85	85	79	87	85	...	53	48	50	34	35	30	34	41
June 23	Sept. 15	Sept. 13	Sept. 9	Sept. 12	Sept. 12	Sept. 10	Sept. 7	Sept. 15	Sept. 12	84	82	78	81	81	79	76	84	81	...	53	47	48	32	33	25	28	38
June 30	Sept. 17	Sept. 15	Sept. 11	Sept. 15	Sept. 14	Sept. 12	Sept. 9	Sept. 17	Sept. 14	79	77	73	77	76	74	71	79	76	...	50	46	44	27	35	24	25	36
July 7	Sept. 18	Sept. 17	Sept. 14	Sept. 18	Sept. 19	Sept. 14	Sept. 14	Sept. 18	Sept. 17	73	72	69	73	74	69	69	73	72	...	47	41	39	23	32	21	22	32
July 14	Sept. 22	...do...	Sept. 15	Sept. 22	Oct. 2	Sept. 13	Sept. 18	Sept. 22	Sept. 20	70	65	63	70	80	61	66	70	68	...	40	36	36	25	29	20	18	29
July 21	Sept. 21	Sept. 21	Sept. 18	Sept. 24	Oct. 3	Sept. 19	Sept. 21	Sept. 30	Sept. 23	62	62	59	65	74	60	62	71	64	...	36	33	34	25	27	20	17	27
July 28	Sept. 29	Sept. 27	Sept. 24	Sept. 28	Oct. 6	Sept. 23	Sept. 23	Oct. 1	Sept. 28	63	61	57	62	70	55	53	65	61	...	33	30	31	24	25	20	16	26
Aug. 4	Oct. 1	Oct. 2	Sept. 29	Oct. 2	Oct. 8	...do...	Oct. 5	Oct. 4	Oct. 2	58	59	56	59	65	50	62	61	59	...	30	25	25	22	23	20	15	23
Aug. 11	Oct. 6	Oct. 7	Oct. 3	.....	.....	Oct. 4	Oct. 8	Oct. 6	Oct. 6	57	58	54	.....	.....	55	59	57	57	...	25	22	22	...	19	20	12	20
Aug. 18	Oct. 16	Oct. 19	Oct. 9	.....	.....	Oct. 7	Oct. 10	.....	Oct. 12	59	62	52	.....	.....	50	53	.....	55	...	23	21	20	...	16	20	...	20

The supply of moisture in the soil available to the plant and the relative humidity are additional factors which conceivably might affect the initiation of reproductive processes. For obvious reasons records of rainfall alone do not furnish a very satisfactory indication of available soil moisture, but for the sake of comparison the total rainfall was computed from Weather Bureau records during the period of vegetative growth of each planting of the Mandarin in 1925, 1926, and 1927, and the data are given in Table 5. Corresponding values of the computed relative humidity also are included in the table. The figures for relative humidity were obtained by averaging the

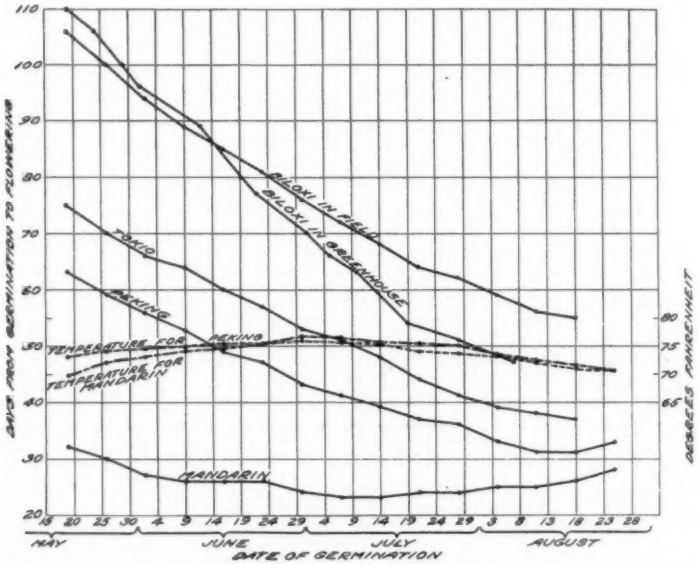

FIGURE 1.—Average number of days from germination to first flowering in field plantings at Arlington Experiment Farm, Rosslyn, Va., of early, medium, and late varieties of soybeans made at short intervals through the growing season in 1919, 1920, and 1922-1927, and in similar greenhouse plantings of the latest variety (Biloxi) in the corresponding season of 1928; also the computed average mean temperature for the vegetative period of each planting of the early (Mandarin) and medium (Peking) varieties. In all except the earliest variety there is a progressive shortening of the vegetative period with advance of season, the maximum effect occurring in the latest variety, Biloxi (see text p. 731)

observations made daily at 8 a. m., noon, and 8 p. m. by the Weather Bureau.

GREENHOUSE PLANTINGS MADE AT INTERVALS THROUGH THE YEAR

Beginning June 18, plantings of Mandarin, Peking, and Biloxi soybeans were made in the greenhouse at intervals of three to five days. The experiment was continued till the middle of August in the following year, a period of about 14 months. An effort was made to maintain an approximately constant mean temperature as far as conditions would permit, but there was no control of the humidity. Facilities were not available for automatic control of the temperature, but it was possible to secure fairly satisfactory results, particularly during

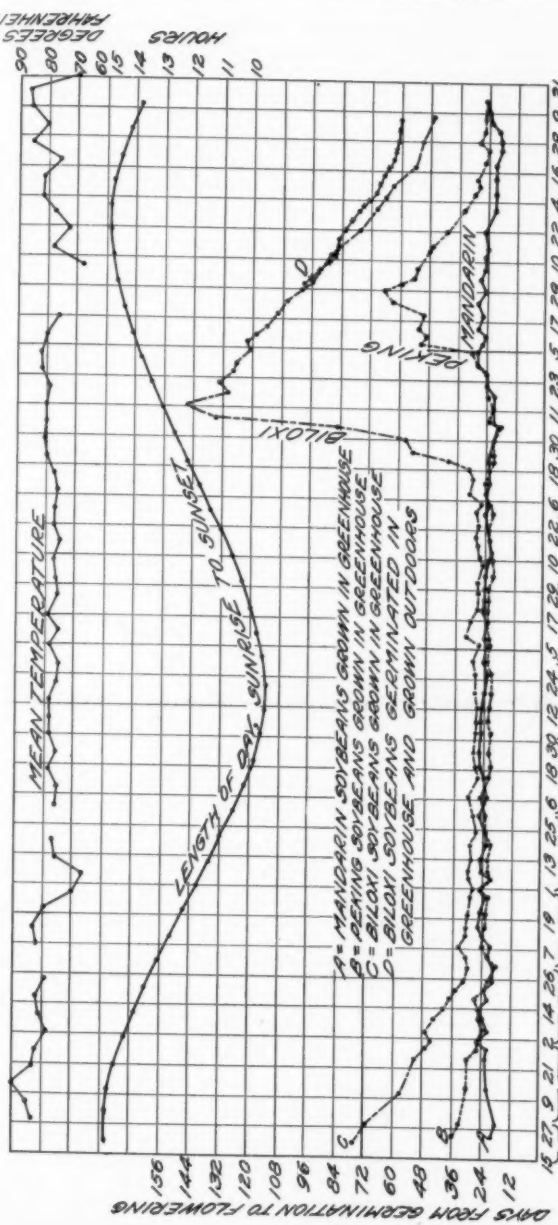
DEGREES FAHRENHEIT
HOURS
MEAN TEMPERATURE
LENGTH OF DAY, SUNRISE TO SUNSET
A = MANDARIN SOYBEANS GROWN IN GREENHOUSE
B = PEKING SOYBEANS GROWN IN GREENHOUSE
C = BILOXI SOYBEANS GROWN IN GREENHOUSE
D = BILOXI SOYBEANS GERMINATED IN GREENHOUSE AND GROWN OUTDOORS
BILOXI
PEKING
MANDARIN
DAYS FROM GERMINATION TO FLOWERING
DAY OF MONTH AND DATE OF GERMINATION
JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY JUNE JULY AUG.

FIGURE 2.—Number of days from germination to first flowering in greenhouse plantings of early, medium, and late varieties of soybeans made at frequent intervals through the year; also the mean weekly temperature in the greenhouse and the seasonal change in length of day. With a favorable, comparatively constant temperature the vegetative period of all varieties was about the same during the fall, winter, and early spring, when the days were short. Later in the spring the increasing day length showed a marked selective action on the different varieties, and only the early variety continued to flower during the longest days of summer (see text, p. 731)

the late fall, winter, and early spring, by hand control of heating and ventilation. Unfortunately, three of the weekly temperature records were lost. In general, the temperature level was close to the mean midsummer temperature at Washington.

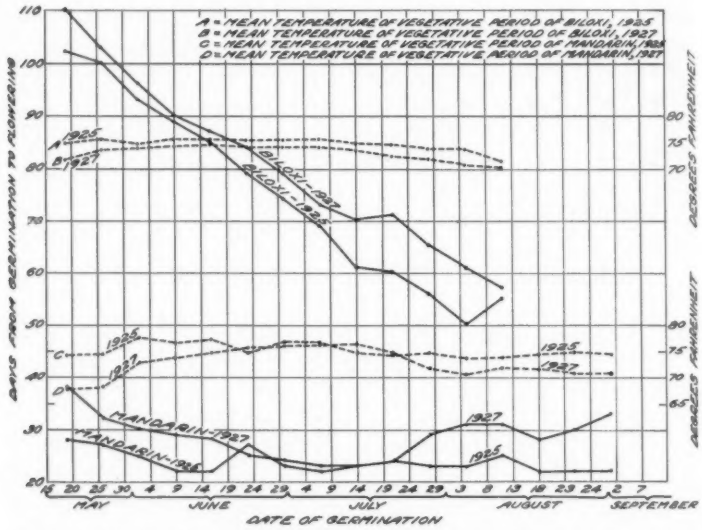

FIGURE 3.—Number of days from germination to first flowering in the successive plantings of the earliest (Mandarin) and the latest (Biloxi) varieties of soybeans in 1925 and 1927; also the computed mean temperature during the vegetative period of each planting of the two varieties. Earlier flowering in 1925 as compared with 1927 in all plantings, except those of the Mandarin made in midsummer, seems to be definitely correlated with the relatively high temperature prevailing in 1925 (see text, p. 730)

TABLE 5.—*Mean relative humidity and total rainfall during the preflowering stage of growth in successive field plantings of Mandarin soybeans at Washington, D. C., in 1925, 1926, and 1927*

Average date of germination	Mean relative humidity in—			Total rainfall in—		
	1925	1926	1927	1925	1926	1927
	Per cent	*Per cent*	*Per cent*	*Inches*	*Inches*	*Inches*
May 19	56	59	68	0.77	1.62	3.26
May 26	59	60	68	1.22	1.73	3.98
June 2	59	61	66	1.51	1.38	3.98
June 9	59	64	65	1.90	2.56	4.09
June 16	63	64	68	2.61	2.41	2.47
June 23	66	64	67	2.46	3.53	5.10
June 30	64	66	70	1.86	4.01	1.18
July 7	65	65	70	3.36	2.52	1.48
July 14	65	69	70	2.92	2.13	2.05
July 21	67	69	68	5.42	1.34	1.38
July 28	67	75	71	3.85	4.72	3.42
August 4	67	76	72	3.24	5.18	4.18
August 11	66	79	73	2.52	8.57	3.16

The results with respect to duration of the vegetative period obtained with each variety are shown by means of graphs in Figure 2. The mean weekly temperatures in the greenhouse and the annual range in

day length, sunrise to sunset, are likewise shown in the form of graphs. To facilitate comparison the results with Biloxi for the period of the year corresponding to that of the field plantings are plotted in part also in Figure 3.

In connection with the above tests, plantings of the Biloxi were made in boxes in the greenhouse at intervals, beginning May 27, and after germination had taken place the boxes were placed out of doors. The time required for each planting to reach the flowering stage, as compared with the results in the greenhouse, is shown in Figure 2, D.

PLANTINGS THROUGH THE OPEN GROWING SEASON EXPOSED TO A 10-HOUR DAY

Beginning April 20, plantings of the Peking and Biloxi varieties were made at intervals of three to five days in large boxes in the greenhouse. After germination the boxes were placed on trucks which were daily wheeled out of doors early in the morning and 10 hours later returned to ventilated dark houses having temperatures approximating those prevailing outdoors. In this way the soybeans were

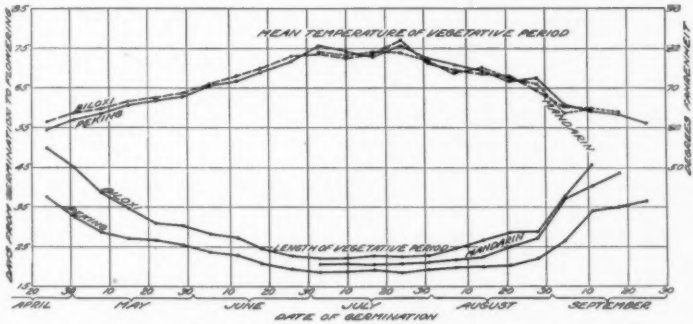

FIGURE 4.—Number of days from germination to first flowering in successive plantings of the Mandarin, Peking, and Biloxi varieties of soybeans which were exposed to approximately outdoor conditions of temperature and a fixed day length of 10 hours. The midsummer temperature appears to be near the optimum for flowering in all varieties, and the lower temperatures of late spring and early fall cause a considerable delay in flowering. There seems to be little indication of a selective action of temperature on the early and late varieties

exposed to a fixed day length of 10 hours but were subjected to essentially outdoor conditions of temperature. Beginning July 1, similar plantings of the Mandarin variety were included in the test. The results as to time required for each variety to reach the flowering stage are shown graphically in Figure 4, which also shows in each instance the computed mean temperature of the vegetative period of plants.

The Weather Bureau records indicate that for the most part the seasonal weather conditions in the year of the test (1928) were fairly close to normal, especially with respect to temperature. The mean temperature for August, however, was 2 degrees above normal and that of September 3 degrees below normal. Except in July the relative humidity averaged somewhat above normal. The rainfall was below normal in June and July but extraordinarily heavy in August and above normal in September. On the whole the weather conditions seem to have been such as to give a very good picture of the average seasonal effect of temperature on soybeans when the factor of length of day is inoperative.

DISCUSSION OF RESULTS

As a basis for interpreting the results obtained in the present series of experiments, it may be regarded as established that the Mandarin, Peking, Tokyo, and Biloxi varieties of soybeans, which range in behavior from very early to very late flowering types when grown in the field in the vicinity of Washington, D. C., all flower in midsummer within a period of 20 to 25 days after germination, if exposed to an artificially shortened day length. In other words, when exposed to a warm summer temperature and a short day, all behave as early varieties. It has been observed also that when grown in a warm greenhouse in the winter months, when the days are naturally short, these varieties show the same behavior. It has been shown in an earlier paper, moreover, that the soybean is a warmth-loving type, with the result that a cool temperature tends to delay flowering. This fact has been further brought out by Eaton [6] and by Gilbert.[7]

In the successive plantings in the field through the season, covering a period of eight years (Tables 1, 2, 3, 4, and fig. 1), there are two features of the growth relations which are of interest: (1) Variations from year to year in growth and in date of flowering of plantings germinating on any given date; (2) a general trend toward reduced growth in all varieties and shortening of the vegetative period in all varieties except the Mandarin as the date of germination is advanced. Despite the rather wide variations from year to year, the general trends with advance of season are clearly evident in each year of the test. With respect to the yearly fluctuations, the results in the years 1925 and 1927 (fig. 3) serve to bring out the significance of variation in temperature as a causal factor. In the Biloxi the vegetative period was longer in 1927 than in 1925 throughout the season. In the Mandarin the same difference occurs except in the midsummer plantings. In both cases there is obviously a close correlation between the differences in mean temperature and the differences in length of the preflowering growth period in the two years. Under the conditions of the experiment, temperature seems to be the dominant factor in the yearly fluctuations in time of flowering. The indications are that sustained temperatures below the midsummer average of about 75° to 77° F. will ordinarily tend to cause a delay in flowering. Moreover, it appears that, under the conditions, a decrease of 1° in the mean temperature of the vegetative period causes a delay of some two or three days in date of flowering.

A comparison of the computed mean relative humidity and the total rainfall during the vegetative period of each of the Mandarin plantings in 1925, 1926, and 1927 (Table 5) with the duration of the period of vegetative activity (Table 1) fails to show any consistent correlation. For example, the longer vegetative period of the 1927 plantings germinating prior to June 23, as compared with the 1925 data, is associated with a higher humidity and increased rainfall. When the 1926 data are included in the comparison, however, it is seen that in all plantings germinating prior to June 23 the dates of flowering in 1926 and 1927 agree closely and are considerably later than in 1925, whereas the humidity records and in large part the

[6] EATON, F. M. ASSIMILATION-RESPIRATION BALANCE AS RELATED TO LENGTH OF DAY REACTIONS OF SOYBEANS. Bot. Gaz. 77: 311–321, illus. 1924.

[7] GILBERT, B. E. THE RESPONSE OF CERTAIN PHOTOPERIODIC PLANTS TO DIFFERING TEMPERATURE AND HUMIDITY. Ann. Bot. [London] 40: 315–320, illus. 1926.

rainfall data for 1926 agree rather closely with those of 1925 but not with those of 1927. Again, during the latter part of the summer the relative humidity was much higher in 1926 than in 1925, but there was no decided difference in the duration of the vegetative period in the soybeans.

A comparison of the average growth rates of the soybeans in 1925 and 1927 shows decided differences except in the earlier germinations of the Mandarin and Biloxi (Tables 1–4), and the relative heights attained by the plants were much reduced in 1927. On the whole, correlation of differences in height of the plants with temperature differences is not consistently maintained, indicating that other potent factors also are operative. As an illustration, it will be noted that the materially lower temperature prevailing in 1927 during the growing period of early plantings of the Mandarin and Biloxi (fig. 3) failed to retard the growth as compared with results in 1925. Considering the average results for the eight years, it is evident that the final heights attained are greatest in the early plantings.

Obviously, the irregularities, or fluctuations, in duration of the vegetative phase of growth at any given period of the year will tend to disappear when average results over a period of years are considered. As a consequence, the graphs showing the changes in duration of the vegetative phase in the different varieties with advance of season (fig. 1) become relatively smooth, thus presenting very clearly defined trends. In this case, in relation to date of flowering, we presumably have, besides the day-length factor, the effect of a relatively uniform seasonal trend in temperature, instead of the more or less sharply fluctuating temperature conditions likely to occur in any single year. In all except the very early variety of soybeans the effects of temperature and length of day on late spring and early summer plantings apparently are additive, both the rising temperature and the decreasing day length favoring earlier flowering with advance of season. In midsummer the average temperature seems to be near the optimum, and only the length of day acts as a major limiting factor. As soon after midsummer as the average temperature begins to fall the two factors of day length and temperature become opposed, the former tending to hasten flowering and the latter to delay it. With the very early variety, Mandarin, neither day length nor temperature is an important limiting factor in misdummer, while in spring and early fall temperature is the only primary factor.

The above-stated considerations relate primarily to the behavior of the different varieties, considered individually. It is of special interest, however, to determine to what extent the sharp contrasts in behavior with respect to earliness shown by the different varieties are really due to the factors of day length and temperature. Under field conditions at Washington these varietal distinctions are always clearly apparent despite irregularities in actual time of flowering induced by temperature fluctuations. The greenhouse plantings through the year furnish a clear picture of the relative effects of day length on the duration of the vegetative period of the different varieties at different seasons of the year when the obscuring effects of varying temperature are largely removed. (Fig. 2.) During the 6-month period in which the day length is about 12 hours or less, the distinction between the varieties with respect to earliness is almost entirely lost, a fact which is in accord with conclusions reached in the

earlier work. However, as has been previously observed, the minimum duration of the vegetative phase in Biloxi consistently remained slightly greater than for Mandarin and Peking.

Beginning about March 20, the increasing day length seems to have brought about a rather sudden change in the behavior of the Biloxi. The duration of the vegetative phase of growth increased rapidly till, in the plantings germinating April 4 and shortly thereafter, individual plants in increasing numbers remained in the vegetative stage throughout the summer and till the return of short days in the early fall. After April 17 all individuals in each planting remained in the vegetative stage till fall and for a time the successive plantings tended to flower on the same date. This behavior of the Biloxi with advance of season results in a graph of characteristic form. (Fig. 2.)

A word of caution may not be out of place here relative to the interpretation to be placed upon the portion of the graph showing the lengthening of the vegetative period in the plantings after March 20. It is not to be inferred that these plantings attained the flowering stage in the indicated number of days as a direct result of exposure to the long days of late spring and early summer. On the contrary, it should be understood that flowering was initiated in these plantings only when the excessively long days had shortened to the required point in late summer. There is nothing to indicate how long vegetative activity would have continued if the longest days of summer had remained in effect for a prolonged period. The comparatively uniform decrease in duration of the vegetative period in successive plantings following the planting that germinated April 12 is due to the fact that all the plants began to flower as soon as a fairly definite decrease in day length had taken place. Available information indicates that this occurred early in August.

Beginning about May 8, after the day length had further increased, the Peking practically duplicated the change in behavior shown earlier by the Biloxi except that the change in duration of the vegetative stage of development was somewhat less abrupt and of less magnitude. As in the case of the Biloxi, the distinctive form of this portion of the graph (fig. 2) indicates response to a definite decrease in day length. In contrast with the Biloxi and the Peking, the Mandarin showed no significant change in duration of the vegetative stage at any time during the spring and summer. It is clear that the annual cycle of day length exercises a distinctly selective action on the different varieties of soybeans. Moreover, with respect to earliness in flowering, the relative positions of the three varieties under comparatively constant conditions of temperature remained the same during the period of late spring and summer as in the field plantings previously considered. During the remainder of the year all behaved as early varieties, as previously stated, the preflowering stage of growth being only from 20 to 30 days.

A direct comparison of the behavior of the Biloxi in the greenhouse and in the field is made in Figure 3. It is apparent that in the later plantings the shortening of the vegetative period is less rapid in the field than in the greenhouse, and the explanation probably is to be found in the higher temperature level in the greenhouse. Further evidence of the retarding action of suboptimum temperature on date of flowering is furnished by the Biloxi plantings made in the green-

house and transferred to outdoor conditions of temperature after germination. (Fig. 2, D.)

It remains to consider the effects of change in temperature with advance of season when the length of day is held constant. (Fig. 4.) The fixed day length of 10 hours which was employed is approximately the optimum for flowering in each of the three varieties. As the maximum mean temperature of the vegetative stage of growth is approached in the midsummer plantings, the duration of the vegetative stage attains the minimum value of 18 to 22 days. The generally convex form of the temperature curves constitutes a close counterpart of the concave form of the curves representing the duration of the vegetative period. Evidently there is a definite correlation of temperature with the duration of the vegetative stage. There is, however, no suggestion of the sort of varietal distinction in behavior with advance of season that was associated with the annual cycle in length of day under conditions of constant temperature. Though the evidence is not conclusive, there is some indication that the lower temperatures of late spring and early fall exert a somewhat selective, retarding action on attainment of the flowering stage as between the Mandarin and Biloxi, on the one hand, and the Peking on the other hand. However, the earliest variety, Mandarin, and the latest variety, Biloxi, seemingly respond in much the same way to changes in temperature; and there is no indication of selective action of this factor on these two varieties of soybeans.

So far as may be inferred from the foregoing data, differences in temperature do not account for the differences in behavior of soybean varieties. Seasonal change in length of day exercises a decided selective action on the different varieties and may well constitute the controlling external factor in the differences in time required by the several varieties to attain the flowering stage when grown under similar conditions. The annual range in length of day in the latitude of Washington, from about 9½ to 15 hours, is not sufficient to affect greatly the time of flowering in the very early variety, Mandarin. It is probable, however, that in higher latitudes having a maximum day length of, say, 16 hours or more, this variety would tend to behave about as the Peking does at Washington; that is, the date of flowering would be delayed by the longest days of summer.

In so far as the relationships existing between the early and late forms of soybeans are applicable to the different forms of other warmth-loving species which normally flower in early and late summer and early fall, it appears that the length-of-day factor may exercise a dominant influence on the relative adaptation of these early and late forms to a given latitude. As the sensibility to lower temperature increases, the more essential it becomes that the plant be able to initiate reproductive processes in response to changes in day length occurring comparatively early in the season, if the plant is to reproduce itself successfully in any given region outside the Tropics. This apparently would tend to enable the plant to escape the destructive action of cold. In this connection it may be noted that the progress of the seasonal change in length of day keeps well in advance of the corresponding stage in the seasonal temperature change. Thus, in the latitude of Washington the maximum and the minimum day lengths of the year occur about a month in advance of the maximum and the minimum mean temperatures. It seems logical to suppose

that, in contrast with the interrelationship of temperature and length of day as regards effects on warmth-loving species like soybeans, the drop in temperature as summer gives way to fall will serve to aid the decreasing length of day in hastening the attainment of the reproductive stage in short-day plants of the cool-loving type.

SUMMARY

In this paper consideration is given to the comparative behavior of early, medium, and late varieties of soybeans, more particularly with respect to duration of the purely vegetative stage of development when planted at frequent intervals through the growing season and when grown under partially controlled conditions. When planted early in the season in the vicinity of Washington, D. C., these varieties, known as Mandarin, Peking, Tokyo, and Biloxi, normally require about 25, 55, 65, and 95 days, respectively, for attaining the flowering stage. All varieties are quite sensitive to cool temperatures.

Data were obtained on field plantings made at intervals through the growing season over a period of eight years. The time of flowering of plantings of a given date varied considerably from year to year, and these variations seem to be closely correlated with yearly fluctuations in the prevailing temperature. Definite correlation of fluctuations in date of flowering with differences in relative humidity and rainfall could not be traced, although these factors may not be entirely without effect on the results. Despite the yearly fluctuations, very definite trends as to change in duration of the vegetative stage with advance of the season can be seen in each variety, all except the Mandarin showing progressive shortening of the vegetative stage. The mean midsummer temperature at Washington (75° to 77° F.) appears to be approximately optimum for all varieties and the suboptimum temperatures of spring and late summer tend to delay flowering in the very early and very late plantings.

In plantings of Mandarin, Peking, and Biloxi made in the greenhouse through the year, with a fairly constant mean temperature approximating that of midsummer, there was a period of about six months in which all three varieties reached the flowering stage in about 25 days after germination and, therefore, behaved as early varieties. During this period the length of day ranged approximately from 9½ to 12 hours and it is evident that the short days showed no selective action on the different varieties. Late in March, however, when the day length had increased further, the late-flowering variety, Biloxi, suddenly began to lengthen its period of vegetative growth until a maximum of 146 days was reached or until there was a return to short days in early fall. Beginning about six weeks later in the spring, the Peking experienced the same sort of lengthening of the period of vegetative growth, although the change was less pronounced. The Mandarin showed no decided change in duration of the vegetative phase of development. Thus the increasing length of day exercised a distinct selective action on the three varieties of soybeans, and the contrasts in behavior of these varieties were essentially the same as those obtained in the field. In general, in the later plantings, the progressive shortening of the vegetative period with advance of season was somewhat less pronounced in the field than in the greenhouse, presumably because of the retarding action of the lower

temperature out of doors. Moreover, plantings transferred at germination from the greenhouse to outdoor temperatures showed a delay in flowering.

In plantings of the three varieties made at intervals through the growing season and exposed to a fixed day length of 10 hours, there was apparently a rather close correlation of the length of the preflowering stage of growth with the mean temperature. In each variety the minimum vegetative period corresponded to the highest mean temperature of midsummer, with appreciable delays in time of flowering associated with the lower temperatures of late spring and early fall. Apparently the lower temperatures affected the earliest variety, Mandarin, and the latest variety, Biloxi, in much the same way, there being no evidence of a definite selective action of the changing temperature on the two varieties.

The available evidence seems to indicate that under field conditions at Washington variations from year to year in date of flowering of both early and late varieties of soybeans when planted on any particular date are due chiefly to differences in temperature, while length of day is the primary external factor responsible for the fact that one variety is always relatively early and another late in attaining the reproductive stage. It seems likely that the relationships existing between the early and late forms of soybeans will apply equally to many other species, although it does not follow, of course, that the differences in behavior of early and late varieties of all species are explainable on the basis of the length-of-day factor.

The variation in growth rate from year to year in the different varieties, which may be quite marked, seems to be due in part to temperature fluctuations and in part to other environmental factors. Over a period of years the average heights attained by the earlier plantings as a rule markedly exceed those attained by later plantings.

GERMICIDAL EFFICIENCY OF SOAPS AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS[1]

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INTRODUCTION

In a previous paper (*6*)[2] the writers reported the results of work on the germicidal efficiency of coconut-oil and linseed-oil soaps and of their mixtures with cresol. In the present paper they report the results of similar work done, for the most part, in connection with investigations designed for a different purpose, which will account for the choice of test organisms and some of the other details of the technic.

MATERIALS AND METHODS

The materials used were as follows:

Cochin coconut oil, saponification value, 254.8.
Castor oil, saponification value, 179.2.
Linseed oil, saponification value, 191.
Oleic acid, U. S. P., neutralization value, 197.
Lauric acid, C. P., melting point, 43° C.
Phenol, U. S. P., melting point, 40.7° C.
Cresol, U. S. P., boiling point, 195° to 205° C.
Cresylic acid, boiling point, 200° to 240° C. (80 per cent above 205°).
N-Hexylresorcinol, boiling point, 253° to 256° C. at 120 mm. Hg., solidification point, 64° C.
Orthophenylphenol, melting point, 57.5° to 58° C.
Sodium hydroxide, C. P., 96 to 98 per cent NaOH.

The neutral soaps of coconut oil, castor oil, and linseed oil were made by saponifying the respective oils with the required amount of carbonate-free sodium hydroxide solutions in closed flasks on a steam bath. The coconut-oil and castor-oil soaps were then concentrated and cooled and the solid soap was flaked and stored in closed containers. The linseed-oil soap was stored in the liquid form. Sodium oleate was made by adding to a definite weight of oleic acid the chemically equivalent quantity of standard sodium hydroxide solution. The mixture was heated in a closed flask overnight, the flask was allowed to cool to room temperature, and the solution was adjusted to the desired strength by the addition of distilled water. Sodium laurate was made by adding to a definite weight of lauric acid the chemically equivalent quantity of standard sodium hydroxide solution and then adjusting the strength of the solution by adding the proper amount of distilled water.

Alkaline soap solutions were made by adding the desired quantity of sodium hydroxide to the neutral soap solution. The term "added alkali" indicates the presence of sodium hydroxide in excess of that required to form neutral soap.

Acid soap solutions were made by dissolving the fatty acid in the heated stock solution of the neutral soap. The term "added acid"

[1] Received for publication July 24, 1930; issued November, 1930.
[2] Reference is made by number (italic) to Literature Cited, p. 747.

indicates the presence of fatty acid in excess of that required to form neutral soap.

The stock solutions of phenol and soap, and of cresol and soap, were usually concentrated solutions containing approximately 50 per cent of phenol or cresol.

BACTERIOLOGICAL WORK

Bacteriological tests were made by the Rideal-Walker technic, modified as described in the writers' previous paper and still further modified by the use of other test organisms and by the use of beef-infusion broth adjusted to pH 7.2 as the culture medium for all the test organisms except *Eberthella typhi*[3] (*B. typhosus*), for which the unadjusted culture medium of Wright was employed. Previous experience having shown that different strains of the same test organism often vary widely in their resistance to disinfectants, tests were made against a number of different strains of each organism. To avoid useless repetition, only results which are considered representative are included in the tables. Where results are given for more than one strain, the purpose is to indicate the variation in resistance of different strains. Results obtained with some of the soaps against *E. typhi* are shown in Table 1.

TABLE 1.—*Germicidal efficiency of phenol and of various soaps against Eberthella typhi*

Disinfectant	Dilution	Added alkali [a]	Organic matter	Results after indicated exposure period of (minutes) [b]						Test organism
				2½	5	7½	10	12½	15	
		Per cent [c]	*Per cent*							
Phenol	1-95			+	+	−	−	−	−	E. typhi No. 1
Coconut-oil soap	1-100			+	−	−	−	−	−	
	1-150			+	+	−	−	−	−	
	1-200			+	+	+	−	−	−	
	1-300	50		+	−	−	−	−	−	
	1-400			+	+	−	−	−	−	
	1-400	100		−	−	−	−	−	−	
	1-600			+	+	+	−	−	−	
	1-800			+	+	+	+	+	+	
	1-30		Skim milk, 50	+	−	−	−	−	−	
	1-40			+	+	−	−	−	−	
	1-50			+	+	+	+	−	−	
	1-120	100		+	−	−	−	−	−	
	1-140		Skim milk, 50	+	+	+	−	−	−	
	1-160			+	+	+	−	−	−	
Linseed-oil soap	1-25			+	+	+	+	−	−	
Sodium oleate	1-10			+	+	+	−	+	+	
Castor-oil soap	1-20			+	+	−	−	−	−	
	1-40			+	+	+	+	+	+	
Phenol	1-95			+	+	−	−	−	−	E. typhi No. 2
Coconut-oil soap	1-30			+	+	−	−	−	−	
	1-40			+	+	+	+	+	+	
	1-400	100		+	−	−	−	−	−	
	1-600			+	+	+	+	+	−	
Castor-oil soap	1-10			+	+	+	+	+	+	
Phenol	1-100			+	+	−	−	−	−	E. typhi No. 5
Coconut-oil soap	1-40			+	−	−	−	−	−	
	1-60			+	+	+	−	−	−	
	1-80			+	+	+	+	+	+	
	1-600	100		+	−	−	−	−	−	
	1-800			+	+	+	+	+	+	
Castor-oil soap	1-10			+	+	+	+	+	+	

[a] Sodium hydroxide in excess of that required to form the soap.
[b] Growth, (+), no growth (−).
[c] Proportion of added to combined alkali.

[3] The bacteriological nomenclature used in this paper is that of the third edition of Bergey's Manual of Determinative Bacteriology (*1*).

It is evident that strain No. 1 is peculiarly sensitive to the germicidal action of coconut-oil soap. Ordinarily the resistance of *Eberthella typhi* to coconut-oil soap is equivalent to that exhibited by strains No. 2 and No. 5. (Table 1.) In a previous paper (*6*) when only *Eberthella typhi* No. 1 was used to test the efficiency of coconut-oil soap, results were obtained which the writers now know to be too high for other strains of that organism. The addition of sodium hydroxide to coconut-oil soap solutions increases their germicidal efficiency and tends to flatten out the differences observed when the neutral soap is used. The various strains, including No. 1, differed but slightly in resistance to phenol.

Results obtained with coconut-oil soap against *Staphylococcus aureus* and various hemolytic streptococci are shown in Table 2.

TABLE 2.—*Germicidal efficiency of phenol and of coconut-oil soap against Staphylococcus aureus and hemolytic streptococci*

Disinfectant	Dilution	Added alkali [a]	Results after indicated period of exposure (minutes) [b]						Test organism
			2½	5	7½	10	12½	15	
		Per cent [c]							
Phenol	1-70		+	+	−	−	−	−	S. aureus No. 1.
Coconut-oil soap	1-5	100	+	+	+	+	+	+	
	1-20		+	+	−	−	−	−	
	1-30		+	+	+	+	+	+	
Phenol	1-75		+	+	−	−	−	−	S. aureus No. 4.
Coconut-oil soap	1-5	100	+	+	+	+	+	+	
	1-30		+	+	−	−	−	−	
	1-40		+	+	+	+	−	−	
Phenol	1-90		+	+	+	−	−	−	Hemolytic streptococcus No. 1.
Coconut-oil soap	1-250		+	+	+	−	−	−	
	1-300		+	+	+	+	+	+	
Phenol	1-90		+	+	+	−	−	−	Hemolytic streptococcus No. 2.
Coconut-oil soap	1-2,000		+	+	+	+	−	−	
Phenol	1-90		+	+	+	+	+	+	Hemolytic streptococcus No. 3.
Coconut-oil soap	1-10		+	+	+	+	+	−	
Phenol	1-90		+	+	+	−	−	−	Hemolytic streptococcus No. 4.
Coconut-oil soap	1-1,000		+	+	−	−	−	−	
Phenol	1-90		+	+	+	−	−	−	Hemolytic streptococcus No. 5.
Coconut-oil soap	1-500		+	+	+	+	−	−	

[a] Sodium hydroxide in excess of that required to form the soap.
[b] Growth (+), no growth (−).
[c] Proportion of added to combined alkali.

Coconut-oil soap in 20 per cent solution did not kill *Staphylococcus aureus* in 15 minutes, but a 5 per cent solution with the addition of 100 per cent excess NaOH killed strain No. 1, the most resistant of the four strains employed, in 7½ minutes.

The results with five strains of streptococci show great differences in resistance to the germicidal action of coconut-oil soap. Somewhat similar variations in the resistance of different strains of streptococci to sodium ricinoleate have been reported by Kozlowski (*3*).

Results obtained with *Salmonella pullorum* and *S. gallinarum* (*Eberthella sanguinaria*) are shown in Table 3.

The results indicate that coconut-oil soap has approximately the same germicidal efficiency against these organisms as against *Eberthella typhi*. Strain 628 of *Salmonella pullorum* was no longer available when the tests were made with castor-oil soap, but the result shown for strain 786 was confirmed by results obtained with three other strains.

TABLE 3.—*Germicidal efficiency of phenol and of various soaps against Salmonella pullorum and S. gallinarum*

Disinfectant	Dilution	Added alkali [a]	Organic matter	Results after indicated period of exposure (minutes) [b]						Test organism
				2½	5	7½	10	12½	15	
		Per ct. [c]	*Per cent*							
Phenol	1-90			+	+	+	−	−	−	S. pullorum No. 628.
Coconut-oil soap	1-20			−	−	−	−	−	−	
	1-30			+	+	+	+	+	+	
	1-500	100		+	+	+	−	−	−	
	1-750			+	+	+	+	+	+	
	1-100		Skim milk, 50.	−	−	−	−	−	−	
	1-150			+	+	+	+	+	+	
	1-50		Feces [d]	−	−	−	−	−	−	
	1-100			+	+	+	+	+	+	
Phenol	1-90			+	+	+	−	−	−	S. pullorum No. 786.
Coconut-oil soap	1-10			+	+	+	−	−	−	
	1-20			+	+	+	+	+	+	
	1-500	100		+	+	+	−	−	−	
	1-750			+	+	+	+	+	+	
Castor-oil soap	1-10			+	+	+	+	+	+	
Phenol	1-100			+	+	+	−	−	−	S. gallinarum.
Coconut-oil soap	1-30	100		+	−	−	−	−	−	
	1-40			+	+	+	+	+	+	
	1-400			+	+	−	−	−	−	
	1-600			+	+	+	+	+	+	
	1-100		Skim milk, 50.	+	−	−	−	−	−	
	1-120			+	+	+	+	−	−	

[a] Sodium hydroxide in excess of that required to form the soap.
[b] Growth (+), no growth (−).
[c] Proportion of added to combined alkali.
[d] 1 gm. dry chicken feces to 5 c. c. of disinfectant.

In Table 4 are shown the results of tests against *Pasteurella avicida* and *P. suiseptica*, indicating that coconut-oil soap has a very high germicidal efficiency against these organisms in the absence of organic matter.

TABLE 4.—*Germicidal efficiency of phenol and of various soaps against Pasteurella avicida and P. suiseptica*

Disinfectant	Dilution	Added alkali [a]	Organic matter	Results after indicated period of exposure (minutes) [b]						Test organism
				2½	5	7½	10	12½	15	
		Per ct. [c]	*Per cent*							
Phenol	1-160			+	+	−	−	−	−	P. avicida No. 1176.
Coconut-oil soap	1-5000			+	+	+	−	−	−	
	1-50		Skim milk, 50.	−	−	−	−	−	−	
	1-100			+	+	+	+	+	−	
	1-1, 000	100		−	−	−	−	−	−	
	1-1, 200			+	+	+	+	+	+	
	1-150		Skim milk, 50.	+	−	−	−	−	−	
	1-200			+	+	+	+	+	+	
Phenol	1-170			+	+	−	−	−	−	P. avicida No. 930.
Coconut-oil soap	1-6, 000	10		+	−	−	−	−	−	
	1-8, 000			+	+	+	+	+	−	
	1-2, 000			+	−	−	−	−	−	
	1-4, 000			+	+	+	+	+	+	
	1-1, 200	100		+	−	−	−	−	−	
	1-1, 400			+	+	+	+	+	+	
Coconut-oil soap	1-2, 000			+	+	−	−	−	−	P. avicida No. 1.
Castor-oil soap	1-1, 250			+	+	+	+	−	−	
Linseed-oil soap	1-1, 500			+	+	+	+	+	+	
	1-20			−	−	−	−	−	−	
	1-40			+	+	+	+	+	+	
Phenol	1-170			+	+	−	−	−	−	P. suiseptica No. 13.
Coconut-oil soap	1-6, 000			+	+	−	−	−	−	
	1-8, 000			+	+	+	+	−	−	
	1-1, 200	100		+	+	+	+	−	−	
	1-1, 600			+	+	+	+	+	+	
Phenol	1-170			+	+	−	−	−	−	P. suiseptica No. 37.
Coconut-oil soap	1-4, 000			+	+	+	−	−	−	
	1-6, 000			+	+	+	+	+	+	
	1-1, 000	100		+	+	+	−	−	−	
	1-1, 200			+	+	+	+	+	+	

[a] Sodium hydroxide in excess of that required to form the soap. [b] Growth (+), no growth (−).
[c] Proportion of added to combined alkali.

The addition of excess sodium hydroxide diminished the efficiency of coconut-oil soap. A decrease in efficiency was caused by as little as 10 per cent excess sodium hydroxide. Organic matter in the form of skim milk also reduced the efficiency of the soap, but this decrease was less when excess NaOH was used. Strain No. 1 is exceptional in its resistance. The limits of variation in resistance of the other seven strains are indicated by the results shown for strains 1176 and 930. It will be seen that when tested against strain No. 1, castor-oil soap had about one-half the germicidal efficiency of coconut-oil soap.

The results shown in Table 5 indicate that the addition of excess lauric acid to sodium laurate increases its germicidal efficiency against *Pasteurella avicida* and a hemolytic streptococcus while excess alkali decreases efficiency. As sodium laurate is the principal constituent of coconut-oil soap these results should be considered in connection with the results shown in Table 4.

TABLE 5.—*Germicidal efficiency of neutral, acid, and alkaline sodium laurate against Pasteurella avicida and hemolytic streptococcus*

Sodium laurate	Added acid or alkali [a]	Results after indicated period of exposure (minutes) [b]						Test organism
		2½	5	7½	10	12½	15	
	Per cent [c]							
1-6, 000		+	+	−	−	−	−	P. avicida No. 3.
1-7, 000		+	+	+	+	+	+	
1-8, 000	Acid, 20	−	−	−	−	−	−	
1-10, 000		+	+	+	+	+	+	
1-1, 000	Alkali, 100	+	−	−	−	−	−	
1-1, 250		+	+	+	−	−	−	
1-1, 500		+	+	+	+	+	+	
1-8, 000	Acid, 10	+	+	−	−	−	−	P. avicida No. 930.
1-9, 000		+	+	+	+	−	−	
1-10, 000		+	+	−	−	−	−	
1-12, 000		+	+	+	+	+	+	
1-12, 000	Acid, 20	+	+	−	−	−	−	
1-14, 000		+	+	+	+	+	+	
1-10, 000	Acid, 40	+	+	−	−	−	−	
1-12, 000		+	+	+	+	+	+	
1-2, 000	Alkali, 100	+	−	−	−	−	−	Hemolytic streptococcus No. 4.
1-2, 400		+	+	+	+	+	+	
1-1, 000		+	+	+	+	+	+	
1-2, 500	Acid, 50	+	−	−	−	−	−	
1-3, 000		+	+	+	+	+	+	

[a] Lauric acid or sodium hydroxide in excess of that required to form sodium laurate.
[b] Growth (+), no growth (−).
[c] Proportion of added to combined acid or alkali.

The effect of the addition of soap on the germicidal efficiency of phenol is indicated by the results shown in Table 6.

As previous work had indicated probable maximum efficiency with 2 parts of phenol to 1 part of soap, this proportion was used. It will be noted that with *Eberthella typhi* and *Salmonella pullorum* as the test organisms the addition of coconut-oil soap or castor-oil soap multiplied the efficiency of phenol from two to three times while linseed-oil soap increased the efficiency only about 50 per cent. With *Staphylococcus aureus* as the test organism the increase in efficiency was about 50 per cent with each of the three soaps. With *E. typhi* as the test organism sodium oleate did not increase the germicidal efficiency of phenol but on the other hand did not decrease it.

TABLE 6.—*Germicidal efficiency of mixtures containing phenol and various soaps*

Phenol dilution	Soap [a]	Organic matter	Results after indicated period of exposure, (minutes) [b]						Test organism
			2½	5	7½	10	12½	15	
		Per cent							
1-70			+	+	−	−	−	−	
1-100	Coconut-oil		+	+	−	−	−	−	
1-120			+	+	+	+	+	+	
1-100	Linseed-oil		+	+	−	−	−	−	Staphylococcus aureus No. 209.
1-120			+	+	+	+	+	+	
1-120	Castor-oil		+	+	−	−	−	−	
1-140			+	+	+	+	−	−	
1-90			+	+	+	−	−	−	
1-320	Coconut-oil		+	−	−	−	−	−	
1-400			+	+	+	+	+	−	Salmonella pullorum No. 685.
1-240	Castor-oil		+	−	−	−	−	−	
1-320			+	+	+	−	−	−	
1-95			+	+	−	−	−	−	
1-300	Coconut-oil		+	+	−	−	−	−	Eberthella typhi No. 1.
1-400			+	+	+	+	+	+	
1-240			+	−	−	−	−	−	
1-320			+	+	+	+	+	+	E. typhi No. 2.
1-160	Linseed-oil		+	+	+	−	−	−	
1-240			+	+	+	+	+	+	E. typhi No. 1.
1-300	Castor-oil		+	−	−	−	−	−	
1-400			+	+	+	+	−	−	
1-240			+	+	−	−	−	−	
1-320			+	+	+	+	+	+	E. typhi No. 2.
1-100	Sodium oleate		+	+	−	−	−	−	
1-150			+	+	+	+	+	+	
1-90	None	Skim milk, 25	+	+	−	−	−	−	
1-95			+	+	+	−	−	−	
1-85	None	Skim milk, 50	+	+	−	−	−	−	E. typhi No. 1.
1-90			+	+	+	−	−	−	
1-120	Castor-oil	Skim milk, 50	+	−	−	−	−	−	
1-160			+	+	−	−	−	−	
1-200			+	+	+	−	−	−	

[a] Concentration of soap is half that of phenol.

[b] Growth (+), no growth (−).

In view of the work of Frobisher (*2*) showing that sodium oleate decreased the germicidal efficiency of phenol, further experiments were made, the results of which are shown in Table 7.

TABLE 7.—*Germicidal efficiency of mixtures of phenol with sodium oleate against Eberthella typhi*

EXPERIMENT 1 [a]

Phenol Dilution	Sodium oleate dilution	Results after indicated period of exposure (minutes) [b]						Test organism
		2½	5	7½	10	12½	15	
1-100		+	+	+	−	−	−	
1-60	1-30	−	−	−	−	−	−	
1-80	1-40	+	−	−	−	−	−	
1-100	1-50	+	+	−	−	−	−	E. typhi No. 1.
1-60	1-15	+	−	−	−	−	−	
1-80	1-20	+	+	+	−	−	−	
1-100	1-25	+	+	+	+	+	−	

EXPERIMENT 2 [c]

		5	10	15	20	30	....	
[d] 1-90		+	−	−	−	−	....	
[d] 1-100		+	+	+	−	−	....	
[d] 1-110		+	+	+	+	+	....	
1-100	½ c. c. 1 per cent solution in each tube	+	+	−	−	−	....	E. typhi No. 2.
1-110		+	+	−	−	−	....	
1-120		+	+	+	−	−	....	
1-130		+	+	+	+	−	....	

[a] Medication temperature 25° C., 0.5 c. c. culture to 5 c. c. disinfectant.

[b] Growth (+), no growth (−).

[c] Medication temperature 20° C., 0.1 c. c. culture to 5 c. c. disinfectant.

[d] 0.5 c. c. additional water in each tube.

In experiment 1 the writers' usual technic was employed, while in experiment 2 an attempt was made to approximate the technic described by Frobisher. The amount of culture was 0.1 to 5 c. c. of disinfectant, the medication temperature 20° C., and the test organism the Hopkins strain of *Eberthella typhi*. It should be noted that in experiment 1 sodium oleate was used in the proportions of twice and four times the amount of phenol.

The results presented in Table 8 indicate that the addition of soaps to higher members of the phenol series increases their germicidal efficiency. In the presence of organic matter (Tables 6 and 8) the effect of soaps upon the germicidal efficiency of phenols is greatly diminished.

TABLE 8.—*Germicidal efficiency of mixtures of soaps with higher phenols against various organisms*

Disinfectant		Dilution of phenols	Organic matter	Results after indicated period of exposure (minutes) [b]						Test organism
Phenols	Soap [a]			2½	5	7½	10	12½	15	
			Per cent							
		1-240		+	−	−	−	−	−	Salmonella pullorum No. 685.
		1-280		+	+	+	+	−	−	
	Linseed-oil	1-320		+	−	−	−	−	−	
		1-400		+	+	+	−	−	−	
	Castor-oil	1-600		+	+	−	−	−	−	
		1-700		+	+	+	+	−	−	
	Coconut-oil	1-700		+	−	−	−	−	−	
		1-800		+	+	+	−	−	−	
		1-180		+	+	−	−	−	−	
		1-200		+	+	+	+	+	+	
	Linseed-oil	1-240		+	−	−	−	−	−	Staphylococcus aureus No. 209.
		1-280		+	+	+	+	−	−	
	Castor-oil	1-300		+	+	+	+	−	−	
		1-350		+	+	+	+	+	+	
	Coconut-oil	1-300		+	+	+	−	−	−	
		1-350		+	+	+	+	−	−	
Cresol		1-280		+	−	−	−	−	−	
		1-300		+	+	−	−	−	−	
	Linseed-oil	1-400		+	−	−	−	−	−	
		1-480		+	+	+	−	−	−	
	Castor-oil	1-700		+	−	−	−	−	−	
		1-800		+	+	+	+	+	−	
	Coconut-oil	1-700		+	+	−	−	−	−	
		1-800		+	+	+	+	+	+	
	None	1-220	Skim milk, 50	+	−	−	−	−	−	Eberthella typhi No. 1.
		1-240		+	+	+	−	−	−	
		1-260		+	+	+	+	+	+	
		1-220	Skim milk, 25	+	−	−	−	−	−	
		1-240		+	+	−	−	−	−	
		1-260		+	+	+	+	−	−	
	Linseed-oil	1-240	Skim milk, 50	+	+	−	−	−	−	
		1-280		+	+	+	+	+	−	
		1-320		+	+	+	+	+	+	
		1-240	Skim milk, 25	+	−	−	−	−	−	
		1-280		+	+	−	−	−	−	
		1-320		+	+	+	−	−	−	
		1-600		+	+	+	+	−	−	
		1-700		+	+	+	+	+	+	
	Linseed-oil	1-600		+	−	−	−	−	−	
		1-700		+	+	−	−	−	−	
	Coconut-oil	1-1100		+	+	+	−	−	−	
		1-1200		+	+	+	+	−	−	
Cresylic acid	Linseed-oil	1-400	Serum, 50	+	+	+	−	−	−	
		1-480		+	+	+	+	+	+	
		1-400	Serum, 25	+	+	+	−	−	−	
		1-480		+	+	+	+	+	−	
	Coconut-oil	1-400	Serum, 50	+	+	−	−	−	−	
		1-480		+	+	+	+	+	+	
		1-480	Serum, 25	+	−	−	−	−	−	
		1-560		+	+	+	−	−	−	
		1-640		+	+	+	+	+	+	

[a] Concentration of soap half that of phenols.
[b] Growth (+), no growth (−).

The results of similar experiments with hexylresorcinol are shown in Table 9.

TABLE 9.—*Germicidal efficiency of mixtures of hexylresorcinol with various soaps*

EXPERIMENT 1

Soap	Ratio, soap to hexylresorcinol	Hexylresorcinol dilution	Results after indicated period of exposure (minutes) [a]						Test organism
			2½	5	7½	10	12½	15	
None		1-10,000	−	−	−	−	−	−	Eberthella typhi No. 1.
		1-12,000	+	+	−	−	−	−	
		1-14,000	+	+	+	+	−	−	
Castor-oil	1:2	1-14,000	+	−	−	−	−	−	
		1-16,000	+	+	+	+	−	−	
		1-18,000	+	+	+	+	+	+	
	1:1	1-12,000	+	−	−	−	−	−	
		1-16,000	+	+	+	+	+	−	
	2:1	1-12,000	+	−	−	−	−	−	
		1-16,000	+	+	+	+	−	−	
Coconut-oil	1:2	1-14,000	+	−	−	−	−	−	
		1-16,000	+	+	+	+	−	−	
		1-18,000	+	+	+	+	+	+	
	1:1	1-12,000	+	−	−	−	−	−	
		1-16,000	+	+	+	−	−	−	
	2:1	1-12,000	+	+	−	−	−	−	
		1-16,000	+	+	+	+	+	+	

EXPERIMENT 2

None		1-12,000	−	−	−	−	−	−	E. typhi No. 1.
		1-14,000	+	+	−	−	−	−	
		1-16,000	+	+	+	+	−	−	
Castor-oil	1:2	1-14,000	+	−	−	−	−	−	
		1-16,000	+	+	−	−	−	−	
		1-18,000	+	+	+	+	−	−	
	2:1	1-14,000	+	−	−	−	−	−	
		1-16,000	+	+	−	−	−	−	
		1-18,000	+	+	+	+	−	−	
	8:1	1-10,000	+	+	−	−	−	−	
		1-12,000	+	+	−	−	−	−	
		1-14,000	+	+	+	−	−	−	
		1-16,000	+	+	+	+	−	−	
		1-18,000	+	+	+	+	−	−	
Sodium oleate	1:2	1-12,000	+	−	−	−	−	−	
		1-14,000	+	+	−	−	−	−	
		1-16,000	+	+	+	+	+	−	
	2:1	1-9,000	+	−	−	−	−	−	
		1-12,000	+	+	+	+	−	−	
		1-15,000	+	+	+	+	+	+	
	4:1	1-6,000	+	+	+	+	+	+	
None		1-4,750	−	−	−	−	−	−	E. typhi No. 2.
		1-5,000	+	+	−	−	−	−	
		1-5,250	+	+	+	+	+	+	
Coconut-oil	1:2	1-5,250	+	−	−	−	−	−	
		1-5,500	+	+	−	−	−	−	
		1-5,750	+	+	+	+	−	−	
		1-6,000	+	+	+	+	+	+	
Castor-oil	1:2	1-5,500	+	−	−	−	−	−	
		1-6,000	+	+	+	+	+	−	
None		1-5,000	+	+	−	−	−	−	Staphylococcus aureus No. 209.
		1-5,500	+	+	+	−	−	−	
		1-6,000	+	+	+	+	+	−	
Castor-oil	1:2	1-6,000	+	−	−	−	−	−	
		1-8,000	+	+	+	+	+	−	
Coconut oil	1:2	1-6,000	+	+	−	−	−	−	
		1-8,000	+	+	+	+	+	+	

[a] Growth (+), no growth (−).

It should be noted that the stock solution of hexylresorcinol used in experiment 2 differed from that used in experiment 1, and comparisons, therefore, should be made only between mixtures prepared from the same stock solution. Castor-oil soap and coconut-oil soap

used in proportions of 1 part of soap to 2 parts of hexylresorcinol and 2 parts of soap to 1 of hexylresorcinol increased the germicidal efficiency of hexylresorcinol. Used in the same proportions sodium oleate did not have this effect. On the contrary, when larger proportions of sodium oleate were used the efficiency decreased. The marked difference in the resistance of strains No. 1 and No. 2 of *Eberthella typhi* to hexylresorcinol has been reported by the writers in a previous paper (*5*).

In Table 10 are shown the results obtained with a number of experimental "germicidal" soaps which were made by adding various phenolic compounds to coconut-oil soap. No. 1 contained 25 per cent of cresol with 50 per cent of soap; No. 2 contained 25 per cent of "cresylic acid" with 50 per cent of soap; No. 3 contained 37.5 per cent of cresol with 37.5 per cent of soap; No. 4 contained 37.5 per cent of "cresylic acid" with 37.5 per cent of soap; No. 5 contained 6 per cent of orthophenylphenol with 24 per cent of soap; and No. 6 contained 9 per cent of orthophenylphenol with 18 per cent of soap. Nos. 1 and 2 were solid, Nos. 3 and 4 were soft pastes, and Nos. 5 and 6 were liquid. Soaps numbered 1, 2, 3, and 4 had a distinct phenolic odor while Nos. 5 and 6 were odorless.

TABLE 10.—*Efficiency of experimental germicidal soaps*

Disinfectant	Dilution	Organic matter	Results after indicated period of exposure (minutes) [a]						Test organism
			2½	5	7½	10	12½	15	
		Per cent							
Cresol	1–175		+	−	−	−	−	−	Staphylococcus aureus No. 209.
	1–200		+	+	+	+	−	−	
Soap No. 1	1–12.5		+	−	−	−	−	−	
	1–16.7		+	+	+	−	−	−	
	1–25		+	+	+	+	+	+	
Soap No. 2	1–12.5		+	−	−	−	−	−	
	1–16.7		+	+	+	−	−	−	
	1–25		+	+	+	+	+	+	
Soap No. 3	1–50		+	−	−	−	−	−	
	1–100		+	+	+	+	−	−	
	1–50	Skim milk, 50.	+	−	−	−	−	−	
	1–100		+	+	+	+	+	+	
Soap No. 4	1–100	Skim milk, 50.	−	−	−	−	−	−	
	1–100		+	+	+	−	−	−	
Soap No. 5	1–6	Serum, 50.	+	+	+	−	−	−	
	1–12		+	+	+	+	+	+	
	1–6	Skim milk, 50.	−	−	−	−	−	−	
	1–12		+	−	−	−	−	−	
Soap No. 6	1–18	Serum, 50.	+	+	+	−	−	−	
	1–36		+	+	+	+	+	+	
	1–18	Skim milk, 50.	+	−	−	−	−	−	
	1–36		+	+	+	+	+	+	
Orthophenylphenol [b]	1–4,000		−	−	−	−	−	−	Eberthella typhi No. 1.
	1–6,000		+	+	−	−	−	−	
	1–8,000		+	+	+	+	+	+	
	1–800	Skim milk, 50.	−	−	−	−	−	−	
	1–1,200		+	+	+	−	−	−	

[a] Growth (+), no growth (−).
[b] Dissolved in 8 parts of coconut-oil soap, dilution expressed in terms of orthophenylphenol.

The orthophenylphenol used in making soaps Nos. 5 and 6 was a high-grade commercial product. A later sample had a very distinct odor due to impurities present, and it would seem that only the higher grade is suitable for the production of an odorless germicidal soap. Results with *Eberthella typhi* in the absence of organic matter

are included in the table in order to indicate the relative value of orthophenylphenol as compared with other disinfectants under the usual conditions of the phenol coefficient test. In view of results already obtained it would seem that the odorless germicidal soap containing orthophenylphenol should prove useful.

DISCUSSION

So far as neutral soaps are concerned, the results presented in this paper confirm and add to those reported by Walker (7). They indicate that coconut-oil soap is the only common soap with significant germicidal activity at room temperature. Against most of the organisms used in this work the germicidal activity of neutral coconut-oil soap was comparatively low even in the absence of organic matter. Against *Pasteurella avicida*, *P. suiseptica*, and some strains of hemolytic streptococci it manifested very high germicidal activity in the absence of organic matter but comparatively low activity in the presence of organic matter in the form of skim milk. It would seem, therefore, that neutral coconut-oil soap is not suitable for the purpose of general disinfection.

In the presence of organic matter coconut-oil soaps containing excess alkali were more active and more uniform in their action than the neutral soaps against the various bacteria tested.

No entirely satisfactory explanation of the germicidal activity of soaps has been worked out as yet. Various writers have attributed it to alkali set free by hydrolysis or to free alkali and unchanged soap combined. Results presented in this paper suggest that while some such explanation may be true for some of the test organisms used in this work, it is not true for all. For bacteria of the Pasteurella group the writers' results indicate that the fatty acid plays an important part. According to McBain (4) fatty acid set free by hydrolysis combines with the soap to form acid soap, so it would appear to be the acid soap rather than the free fatty acid which has such high germicidal activity against bacteria of the Pasteurella group.

Just as there is no satisfactory explanation for the germicidal activity of soaps so also there is no satisfactory explanation of their action in enhancing the germicidal activity of phenols. Considered from the practical point of view, however, it is plain enough that if a soap is to be used with a phenol it is important to use the right soap and the correct proportion. Results presented in this paper indicate that coconut-oil soap and castor-oil soap are suitable for such use in the proportion of 1 part of soap to 2 parts of phenol.

SUMMARY

Bacteriological tests were made with coconut-oil soap, linseed-oil soap, and castor-oil soap, and mixtures of these with sodium hydroxide or with phenols, against *Eberthella typhi*, *Staphylococcus aureus*, hemolytic streptococci, *Salmonella pullorum*, *Pasteurella avicida*, *P. suiseptica*, and *Salmonella gallinarum*. Some tests were also made with sodium laurate and sodium oleate.

Neutral coconut-oil soap in a concentration of 10 per cent was effective against all the test organisms except *Staphylococcus aureus*. A 20 per cent solution failed to kill *S. aureus* in one hour, while on the

other hand *Pasteurella avicida*, *P. suiseptica*, and some strains of hemolytic streptococci were killed by concentrations varying from 1–1,000 to 1–6,000 within 15 minutes in the absence of organic matter. In the presence of 50 per cent skim milk a concentration of 1–100 was required. Against *P. avicida* neutral linseed-oil soap was effective in 5 per cent concentration, while neutral castor-oil soap was effective in a concentration of 1–1,250, in both instances without organic matter. These two soaps were not tested against *P. suiseptica* but against the other test organisms they were not effective in 10 per cent concentration with 15 minutes exposure.

The addition of sodium hydroxide to solutions of neutral coconut-oil soap made these solutions more effective against all of the test organisms except *Pasteurella avicida*, *P. suiseptica* and some strains of hemolytic streptococci, in which cases efficiency was diminished. The presence of excess alkali decreased the efficiency of sodium laurate against *P. avicida*, while excess lauric acid increased it.

Coconut-oil soap, castor-oil soap, or linseed-oil soap when mixed with various phenolic compounds in the proportion of 1 part of soap to 2 parts of phenol considerably increased the germicidal efficiency of the phenols in the absence of organic matter. In the presence of milk or blood serum such increases were comparatively small.

Germicidal soaps made by adding cresol, commercial cresylic acid, or orthophenylphenol to coconut-oil soap were found to be efficient against *Staphylococcus aureus*, even in the presence of milk or blood serum. The soap containing high-grade orthophenylphenol was odorless.

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VITAMINS IN SUGARCANE JUICE AND IN SOME CANE-JUICE PRODUCTS [1]

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INTRODUCTION

In addition to the commonly accepted food value of sugarcane sirup and molasses as carbohydrates and their place in supplying characteristic flavors and variety in the diet, it was formerly believed in certain sections where cane sugar was manufactured that the juice of the cane was particularly healthful and even had some remedial properties. In the early days of the sugar industry in this country the sugar mill was regarded as a very healthful place to work. The evaporation of the cane juice was carried out in large open cauldrons or kettles, and the steam and vapors escaped into the building. The breathing of these vapors was considered beneficial. Large quantities of the cane juice were drunk, and other products of the sugar factory were included in the diet. Whether the reported beneficial results were real or fancied, however, they contribute an added interest to the study of the vitamin content of sugarcane juice.[3]

As far as the authors are aware but little work has been done on the vitamin content of fresh sugarcane juice. Delf [4] found that sugarcane juice had no antiscorbutic value.

In this paper are presented the results of some experiments carried out to determine the vitamin B [5] and vitamin D potency of the juice of sugarcane. Some preliminary tests were also made for vitamin A. The juice used was obtained from three lots of sugarcane stalks shipped at widely different times and from different sections of the country.

In addition to estimating the vitamins in the juice obtained by crushing whole cane stalks in a laboratory-size sugarcane mill, a comparison was made of the vitamin B value of the juice thus expressed with that obtained by subjecting the bagasse from the small mill to the much higher pressure of a hydraulic press. Comparison was also made of the vitamin B potency of juice obtained from different parts of the same cane stalks. For this purpose juice

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[2] The authors wish to acknowledge their indebtedness to H. S. Paine and R. T. Balch, of the Carbohydrate Division of this bureau, for the analyses of the samples of the cane juice used in this investigation and for supplying the samples of the cane-juice products studied; also to J. F. Brewster for having some of the sugarcane collected and shipped, and to J. P. Divine for technical assistance in carrying out the experimental work.

[3] The writers are indebted for the above information to C. A. Browne, at whose suggestion the work described in this paper was undertaken.

[4] DELF, E. M. STUDIES IN EXPERIMENTAL SCURVY, WITH SPECIAL REFERENCE TO THE ANTISCORBUTIC PROPERTIES OF SOME SOUTH AFRICAN FOODSTUFFS. Lancet 202: 576–578. 1922.

[5] Inasmuch as some of the data presented in this paper were obtained before the multiple nature of the water-soluble vitamin B fraction was recognized, the term "vitamin B" has been used in the old sense to refer to the vitamin B complex.

obtained from the upper third and lower third portions of the cane was used. Several products manufactured from sugarcane juice were also examined. These included cane sirup, different samples of blackstrap molasses, and cane cream.

EXPERIMENTAL DATA

VITAMIN B IN SUGARCANE JUICE

The juice used for these experiments was obtained from sugarcane of the 1926 crop, shipped in cold storage from Florida. As soon as possible after arrival the canes were crushed in a laboratory-size sugarcane mill, and the juice obtained was filtered through two thicknesses of cheesecloth. The fairly clear juice was then distributed into a large number of small wide-mouth bottles and placed in cold storage at a temperature below freezing. The frozen juice was taken out of one bottle at a time as needed and was allowed to liquefy at room temperature. The quantities required daily for the feeding experiments were measured out by means of a pipette.

A sample of the freshly expressed juice was analyzed in the carbohydrate division of this bureau. The results are as follows:

Brix, corrected to 20° C	degrees	14. 64
Direct polarization, 20° C	do	9. 73
True sucrose (invertase method)	per cent	9. 45
Solids (by drying)	do	13. 17
Invert sugar (Meissl and Hiller method)	do	4. 13
Apparent purity, $9.73 \times 100 \div 14.64$	do	66. 46
True purity, $9.45 \times 100 \div 13.17$	do	71. 76

The juice was tested for vitamin B by both the curative and the prophylactic methods. In the curative tests young albino rats were fed a vitamin B free ration consisting of casein, 20 parts; Osboren and Mendel's Salt Mixture IV, 5 parts; butterfat, 15 parts; and cornstarch, 60 parts. When evidence of vitamin B deficiency was manifested the cane juice was fed at definite levels daily apart from the basal ration. The rats were kept in individual cages having raised screen bottoms. The prophylactic tests were similarly conducted except that the cane juice was fed from the start. The results are shown graphically in Figure 1. Lot 1 consisted of two rats used for controls. Each curve of the other four lots represents the average weight of four rats fed the quantities of juice indicated on the chart. The rats of lots 2 and 3 were given the juice from the beginning of the experiment. In the curative tests with rats of lots 4 and 5, the period during which the juice was fed is indicated in each curve by the broken line.

On account of the difficulty of getting the animals of lot 2 to take as much as 20 c. c. of juice daily, the juice was concentrated to one-fourth of its original volume by evaporation at low temperature under diminished pressure. Of this product 5 c. c. was fed daily. From the results shown it appears that 15 c. c. of the cane juice fed daily fell short of supplying enough vitamin B to maintain weight, but that 20 c. c. was sufficient to promote growth at a fair rate for at least 60 days. Some of the rats of each lot did not always take all the juice given them, but it is believed that this did not significantly affect the final average results.

A previous shipment of cane of the 1924 crop had been received from Louisiana. This cane was superior in size, appearance, and in content of juice to that obtained from Florida. On account of unavoidable circumstances it was not possible to study the vitamins in the juice of this cane to the extent that had been planned. However, the curve in Figure 2, which represents the average weight of four

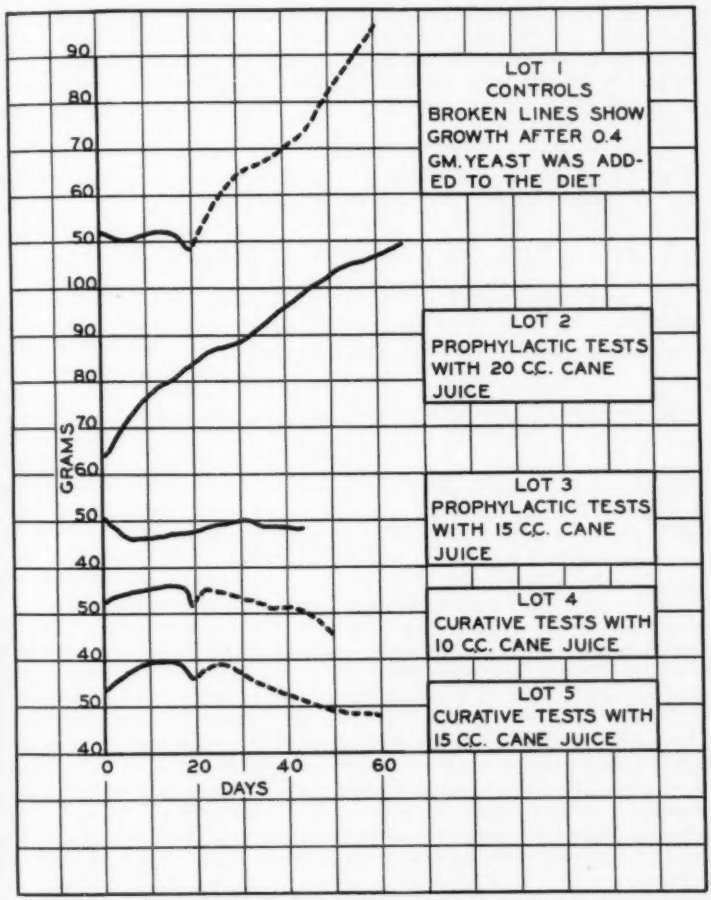

FIGURE 1.—Growth of rats when fed prophylactic and curative doses of cane juice as sources of vitamin B as compared with that of rats fed yeast

rats, shows that the juice from this lot of cane contained more vitamin B than did that of the cane obtained from Florida. Ten cubic centimeters daily of the juice was sufficient to enable the rats to resume growth with an average gain in weight of 15 gm. in 35 days, as indicated by the broken line in the curve. The composition of the basal diet was as follows: Meat residue, 18 gm.; Salts IV, 5 gm.; butterfat, 15 gm.; cornstarch, 62 gm.; making a total of 100 gm.

VITAMIN D IN SUGARCANE JUICE

Tests for vitamin D were made on the juice from the 1926 Florida sugarcane (previously referred to) by means of the "line test".[6] One lot of four rats was brought to a rachitic condition by feeding them for 25 days Steenbock's rachitic ration No. 2965, consisting of yellow corn, 76 parts; wheat gluten, 20 parts, calcium carbonate, 3 parts; and sodium chloride, 1 part. The animals were then given daily 10 c. c. of the cane juice for 15 days. At the end of the period the degree of calcification induced in the radii of the rachitic animals was observed. A trace of calcification of the rachitic metaphyses was noted in the case of one rat. The others gave negative results.

VITAMIN A IN SUGARCANE JUICE

Preliminary tests for vitamin A indicated the presence of but little of this vitamin. Consequently, these studies were not continued further.

VITAMIN B IN SUGARCANE JUICE EXPRESSED AT DIFFERENT PRESSURES

The juice used in the following experiments was obtained from sugarcane of the 1927 crop sent from Franklin, La. Before shipment the ends of the stalks were paraffined partially to protect them from deterioration. The shipment included 100 pounds each of stalks representing the upper third and lower third portions of the cane and 200 pounds of whole cane stalks.

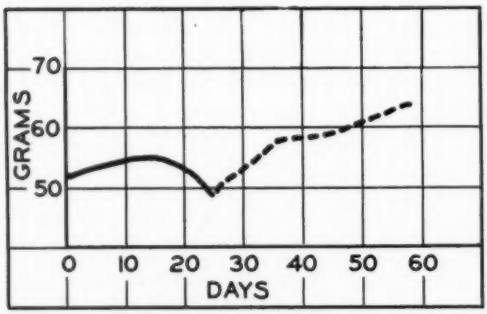

FIGURE 2.—Growth of rats when fed Louisiana cane juice of the 1924 crop as a source of vitamin B

Preliminary to the feeding experiments described later it was desired to ascertain the behavior of the experimental rats on the basal ration used when different levels of yeast furnished vitamin B. The results obtained may also serve as a basis of reference by which the responses of the animals to the sugarcane products tested in the experiments hereafter described can be compared. The vitamin B free basal ration consisted of casein, 18 parts; Osborne and Mendel's Salt Mixture IV, 4 parts; agar, 2 parts; cod-liver oil, 2 parts; and dextrin, 74 parts.

Figure 3 shows the growth response of the animals to different levels of yeast fed as a source of vitamin B. Each curve represents the average weight of four male rats. Although 0.4 gm. of yeast per day permitted growth at a rapid rate, an increased amount of yeast produced better growth. The quantity fed daily is indicated on the chart. On the basal diet used control rats receiving no vitamin B usually

[6] McCOLLUM, E. V., SIMMONDS, N., SHIPLEY, P. G., and PARK, E. A. STUDIES ON EXPERIMENTAL RICKETS. A DELICATE BIOLOGICAL TEST FOR CALCIUM-DEPOSITING SUBSTANCES. Jour. Biol. Chem. 51: 41-49, illus.

cease to gain in weight about the tenth day, and almost invariably lose weight rapidly after the fifteenth day.

Ten kilograms of the whole cane stalks were put through a laboratory-size sugarcane mill. The bagasse (5,150 gm.) was cut rather fine in a feed cutter, and pressed in a large Bushnell press, without use of maceration water, until all the juice that could be expressed was removed. The residual bagasse was then further pressed in a hydraulic press up to a pressure of 2,400 pounds per square inch. A total extraction of 70.6 per cent was obtained, of which 4.8 per cent was removed by the hydraulic press. As it was desired to compare only the juice expressed in the roller mill with that obtained from the hydraulic press, the intermediate fraction removed by the Bushnell press was discarded. The two fractions of the juice were filtered through two thicknesses of cheesecloth, and concentrated by evaporation under reduced pressure to one-fourth of the original volume. The concentrated product was stored at low temperature. The results of analyses of the high and low pressure juices are presented in Table 1.

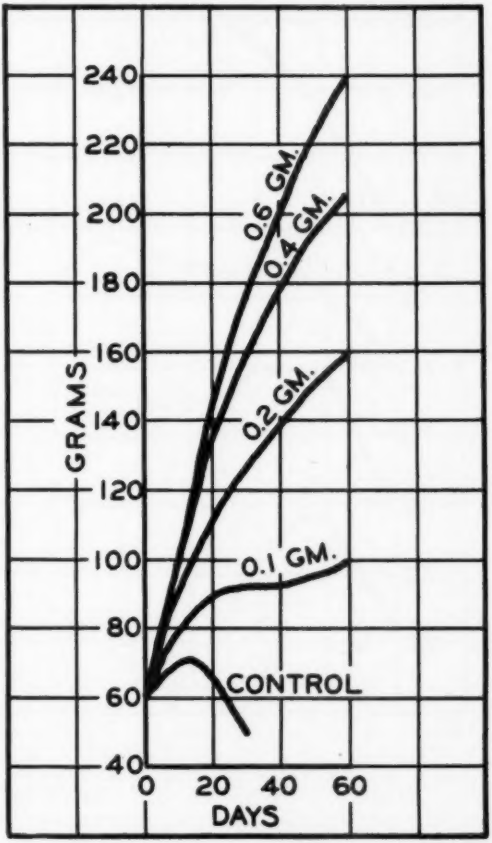

FIGURE 3.—Growth response of rats to different levels of yeast fed as a sole source of vitamin B

As shown in Figure 4, the vitamin B content of the juice obtained at the higher pressure was much greater than that of any other sugarcane juice tested. Each curve represents the average weight of four male rats fed daily, apart from the basal ration, quantities of the concentrated product corresponding to the number of cubic centimeters of the original juice indicated at the right of the chart. The curves of lots 14 and 15 show that the high-pressure juice is about twice as potent in vitamin B as the other juice. A comparison of the curves in this chart with those in Figure 3 shows that 10 c. c. of the high-pressure juice was approximately equal to 0.15 gm. of dried yeast as a source of vitamin B.

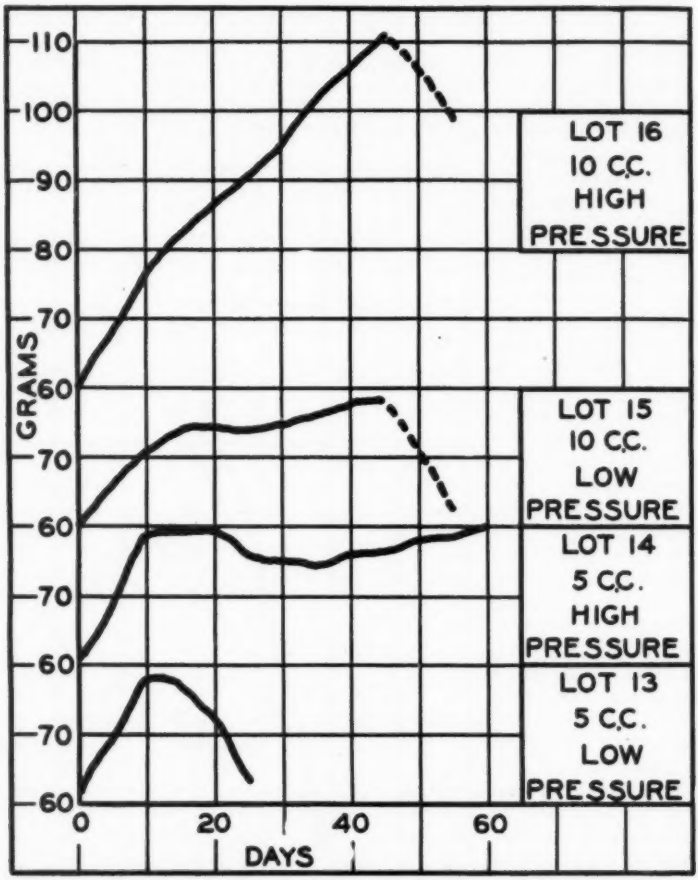

FIGURE 4.—Growth of rats when fed two levels of cane juice expressed at different pressures as a source of vitamin B

TABLE 1.—*Analyses of cane juice expressed at high and low pressure*

Item		Cane juice	
		High pressure	Laboratory cane mill
Brix	degrees	18.60	19.40
Apparent purity	per cent	71.23	80.89
Direct polarization	degrees	13.40	15.71
Invert polarization	do	−3.92	−4.77
True sucrose	per cent	13.95	15.60
Solids (by drying)	do	17.83	19.01
True purity	do	78.24	82.06
Reducing sugars as invert	do	2.40	1.65

Two rats in lot 13 developed severe polyneuritis at 31 and 38 days, respectively. This symptom of vitamin B deficiency, however, was not observed in the other groups. Further evidence that growth in these experiments was not limited primarily by a lack of the pellagra-preventive factor is presented in the curves of lots 15 and 16. At the end of 45 days when the supply of high-pressure juice was exhausted, these lots received 5 per cent of autoclaved yeast. If the diet of these rats had contained an adequate supply of vitamin B, and growth had been limited by the pellagra-preventive factor, growth at the same rate as that shown for the first 10 days of the experimental period would be anticipated when the autoclaved yeast was added to the diet.

VITAMIN B IN SUGARCANE JUICE FROM THE UPPER AND LOWER THIRDS OF THE CANE STALKS

The upper and lower third portions of the cane stalks (100 pounds each) were run through the laboratory cane mill, and the juice was filtered through two layers of cheesecloth in order to remove finely divided particles of stalk. The top portions yielded 11,840 c. c. of juice, and the lower portions 16,880 c. c.

The juice was concentrated and stored as already described. Analysis of the juice gave the results shown in Table 2.

TABLE 2.—*Analyses of juice obtained from the upper and lower third portions of sugarcane*

Item	Juice from upper third	Juice from lower third
Brix.....degrees..	16.5	19.9
Apparent purity.....per cent..	81.0	90.0
Reducing sugars as invert sugar.....do....	1.4	0.5
True sucrose.....do....	14.04	18.13
Solids (by drying).....do....	16.73	19.58
True purity.....do....	83.92	92.60
Direct polarization.....degrees..	+13.55	+17.93
Invert polarization.....do....	−4.91	−5.91

As shown in Figure 5, the upper part of sugarcane stalks contains more vitamin B than the lower part of the same stalks. The animals of lots 26 and 28 received juice from the lower third of the stalks, and lots 27 and 29 received juice from the upper third. Each curve represents the average weight of four male rats fed the daily dose indicated at the right of the chart. One rat in lot 26 and two rats in each of lots 27 and 28 failed to survive the experimental period of 60 days. Seven cases of polyneuritis were observed, as follows: In lot 26, two at 38 and 60 days; in lot 27, two at 45 and 48 days; in lot 28, two at 53 days, and one at 56 days.

VITAMIN B IN SUGARCANE CREAM

Sugarcane cream is a new product developed by the carbohydrate division of this bureau.[7] It is prepared by evaporating cane sirup in an open kettle to a high predetermined density, judged by its boiling temperature; cooling this sirup and crystallizing a portion of

[7] PAINE, H. S., and BALCH, R. T. SUGAR-CANE CREAM A NEW PRODUCT OF COMMERCIAL VALUE. U. S. Dept. Agr. Yearbook 1927: 605–607. 1928.

the sugar therein with continuous stirring with a "cream beater" similar to that used in making sugar fondant for coated confectionery; and warming slightly the finished cream in a jacketed remelt kettle, in order that the cream may flow into the containers of tin or glass.

Examination of this product for vitamin B was conducted as in the preceding experiments. The cane cream was fed daily apart from the basal diet at the levels indicated in Figure 6. Each curve represents the average weight of four rats. The results show that cane

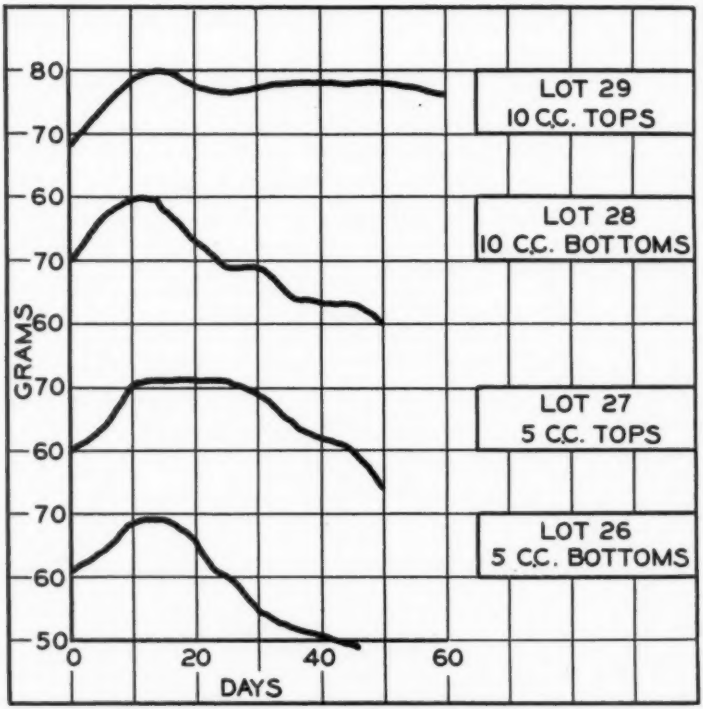

FIGURE 5.—Growth of rats when fed cane juice obtained from different portions of the stalk, as a source of vitamin B

cream contains no vitamin B. The average survival of four male rats in lot 6 was 34½ days; in lot 7, 32 days; and in lot 8, 34 days. Three cases of polyneuritis occurred in lot 6, three in lot 7, and one in lot 8.

VITAMIN B IN SUGARCANE SIRUP AND BLACKSTRAP MOLASSES

Sugarcane sirup and blackstrap molasses were also obtained from the carbohydrate division. The cane sirup had been prepared from sugarcane juice by the Georgia open-pan process. Both Louisiana and Porto Rico blackstrap molasses were examined. Instead of being fed apart from the basal ration as in all the preceding experiments, these products were mixed with dextrin and incorporated in the

ration. The ration was the same as that previously used except that the sirup and molasses replaced an equal weight of dextrin. The average daily intake per animal of the products tested is shown in Table 3, and the growth of the rats fed these products is shown in Figure 7. Each curve in this figure represents the average weight of four male rats. That the products tested did not contain significant amounts of vitamin B is apparent from the fact that no appreciable prolongation of life resulted from their addition to the diet. No case of polyneuritis was observed in lot 56, but in each of the other lots two cases were noted.

TABLE 3.—*Average daily intake per animal of cane products tested*

Item	First week	Second week	Third week	Fourth week
	Gram	*Gram*	*Gram*	*Gram*
Cane sirup	0.41	0.43	0.33	0.24
Porto Rican molasses, 10 per cent	.97	.96	.63	.37
Porto Rican molasses, 5 per cent	.48	.56	.33	.28
Louisiana blackstrap	.54	.53	.29	.26

DISCUSSION

Probably the most important practical application of these findings is concerned with the use of cane molasses as a food for both human and animal consumption. During the last 15 or 20 years immense quantities of molasses have been used in the manufacture of so-called molasses feeds. From the results of the earlier work of Nelson, Heller, and Fulmer[8] it is concluded that there are large variations in the vitamin B content of molasses, since the blackstrap molasses which they investigated was found to be an excellent source of this vitamin, whereas the writers found vitamin B to be absent from two samples of this product. The results obtained in the present experiments show that the inclusion of generous amounts of molasses in the diet does not insure an adequate amount of vitamin B.

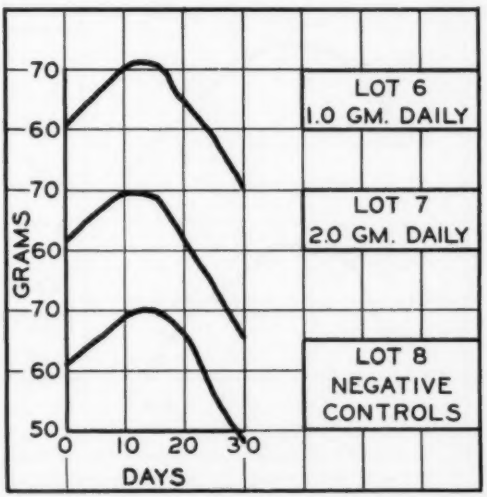

FIGURE 6.—Growth of rats when fed cane cream at different levels as a source of vitamin B

The fact that the upper portion of the cane stalk is richer in vitamin B than the lower portion may be associated with the difference in the

[8] NELSON, V. E., HELLER, V. G., and FULMER, E. I. MOLASSES AS A SOURCE OF VITAMIN B. Indus. and Engin. Chem. 17: 199–201, illus. 1925.

stage of maturity of the different parts of the stalk. The older portion has become a storage organ while the younger portion is still a seat of considerable metabolic activity.[9] While synthesis of sugar takes place in the leaves and these carbohydrates are then translocated to the stalk, changes in the nature of the sugar in the stalk continue until the stalk reaches maturity.

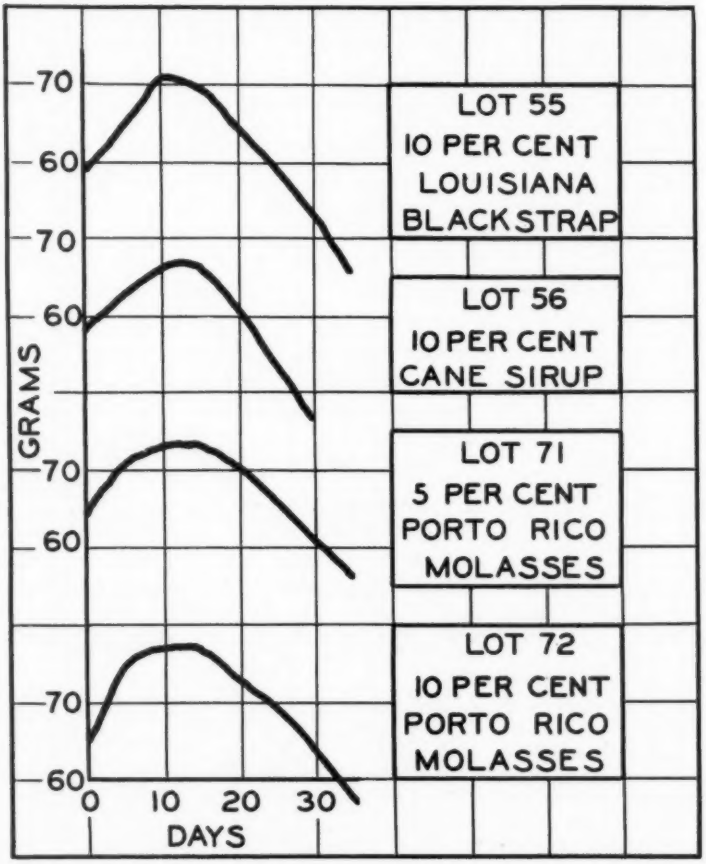

FIGURE 7.—Growth of rats when fed various cane-juice products as sources of vitamin B

Probably the contents of the thin-walled parenchymal cells which contain the sugar are most easily expressed so that the ordinary sugarcane juice does not contain a large portion of other protoplasmic material. By applying much higher pressure the protoplasm of other cells was expressed. The sugar is reserve food for the young plants which develop from the nodes when the stalk comes in contact

[9] DEERR, N. CANE SUGAR: A TEXTBOOK ON THE AGRICULTURE OF THE SUGAR CANE, THE MANUFACTURE OF CANE SUGAR, AND THE ANALYSIS OF SUGAR-HOUSE PRODUCTS. Ed. 2, rev. and enl., 644 p., illus. London. 1921.

with the ground or is buried in it. It seems that there is more vitamin B in those cells in which there is the greatest metabolic activity.

SUMMARY

The results of studies of the vitamin content of sugarcane juice and some commercial products made from it are reported.

Sugarcane juice is a poor source of the antineuritic vitamin. The juice from the upper portions of cane stalks is richer in this vitamin than juice from the lower portions. Juice obtained from bagasse by using high pressure is richer in the antineuritic vitamin than ordinary cane juice.

Sugarcane juice contains a small amount of vitamin A and little, if any, vitamin D.

Cane sirup, Louisiana and Porto Rico blackstrap molasses, and cane cream, products made from sugarcane juice, were found to be devoid of demonstrable quantities of vitamin B.

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